THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XVIII NOVEMBER, 1942 NUMBER 6

BENIGN CHONDROBLASTOMA OF BONE *
A REINTERPRETATION OF THE SO-CALLED CALCIFYING OR CHONDROMATOUS GIANT CELL TUMOR

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Our comprehension of the lesion under discussion developed out of a study of the question of giant cell tumor of bone. That study [1] led us to a rather strict definition of what should be regarded as representing the latter lesion. Collaterally we were led to reject from the category of giant cell tumor, as independent entities, a number of other lesions usually interpreted as modifications or variants of this tumor. Among these rejected lesions is the one under consideration here, which has usually been described as the "benign calcifying" or the "epiphyseal chondromatous" giant cell tumor. We hold that this lesion is a specific type of tumor, developing from cells best interpretable as cartilage germ cells because of the aura of cartilage about the lesion as a whole, and we wish to designate the tumor as "benign chondroblastoma of bone."

This lesion presents certain special clinical features, but these are overshadowed in interest by its anatomic and particularly by its histologic peculiarities. The basic cells of the lesion are polyhedral cells of moderate size which may be closely compacted or somewhat separated by a small amount of interstitial ground substance. But what is striking and specific about the histologic picture is, in the first place, the presence of focal areas of calcification and the fact that where the cells and ground substance have become heavily encrusted with calcium the tissue is necrotic. Furthermore, in the necrotic areas one sees evidence of collagenization, resorption of the necrotic calcified débris and replacement of it by a hyaline chondroid tissue. The latter tissue itself may show patchy calcification and, in some places, even direct metaplasia into bone. This sketch of what we hold to be the distinctive pathologic features of the lesion omits various other aspects of the

* Received for publication, February 13, 1942.

picture and, particularly, mention of the multinuclear giant cells, to which so much importance has been attached in the past. It is true that multinuclear giant cells are to be found, either singly or in small collections, here and there in the lesion, but most of them are seen around areas of hemorrhage, necrosis and reconstruction and we think they should hence not be regarded as part of the primary pattern of the lesion.

It was Ewing [2] who denoted as the "calcifying giant cell tumor" the lesion we are discussing here. He was certainly correct in pointing out that it was not an ordinary giant cell tumor, and that the lesion is a benign one though it has often been mistaken for a malignant one and specifically for an osteogenic sarcoma. However, it was Codman [3] who presented what seems to be the first detailed discussion of the lesion. His paper, entitled "Epiphyseal Chondromatous Giant Cell Tumors of the Upper End of the Humerus," was based on nine cases culled from the Registry of Bone Sarcoma. Codman, too, was led by clinical, roentgenographic and anatomic uniformities to regard the lesion as an entity, though like Ewing he held that this entity belonged within the category of giant cell tumor. Again like Ewing, Codman stressed the predilection of the lesion for the upper end of the humerus and specifically for the vicinity of its tuberosities. However, he did not believe that it was necessarily limited to this region, and, in a more recent reference to the lesion, Ewing [4] also expressed this opinion and seems to imply that it ought no longer to be considered as a form of giant cell tumor.

Otherwise the literature on the subject is very meager. One of the cases included in Codman's [3] report had previously been reported by Geschickter and Copeland [5] as a chondroblastic sarcoma.* Another had been reported by Phemister [6] as a chondrosarcoma. There can be no doubt that the case which King [7] reported as "an example of benign osteogenic sarcoma" likewise represents an instance of the lesion.

We ourselves have studied nine examples of the condition from the records of our laboratory, and these cases, with the pertinent clinical data, constitute the foundation of the present discussion. Like Codman,[3] we find that the condition is a very distinctive one, that the

* Since submitting the manuscript for publication, we have come across an instance of the lesion in the upper end of a tibia which Hammarström,[5a] too, reported as a chondroblastic sarcoma. This case was subsequently re-evaluated by Bergstrand [5b] (from whose laboratory it originally came). He felt that the original designation "chondroblastic sarcoma" was incorrect and thought that the tumor represented a vascular tissue growth but did not state whether or not he still regarded the lesion as malignant. On the other hand, Copeland [5c] continues to refer to the lesion as a chondroblastic sarcoma, and specifically as "a rare osteolytic variant of osteogenic sarcoma, sometimes erroneously called cartilaginous giant cell tumor."

lesion tends to be relatively small, that it is apparently seen only in young subjects and that it yields readily to conservative treatment (curettage with or without subsequent irradiation). Unlike Ewing [2,4] and Codman, however, we hold that, while starting its development in epiphyses and specifically in those of long bones, it does not exhibit predilection for the humerus. Furthermore, as already noted, we do not hold that the lesion, though distinctive, is to be regarded as a variant of giant cell tumor of bone, and believe instead that it is a peculiar tumor having a chondroblastic basis. Our discussion will be devoted mainly to an elaboration of this conception.

Clinical Features

Age and Sex Incidence and Localization

In all nine cases which we studied the patients were males. In Codman's [3] series of nine cases, six were males. The ages of our patients ranged within the narrow limits of 13 and 17 years and averaged 15.5 years. The ages of Codman's patients ranged between 12 and 24 years and averaged 17 years. These data indicate that it is in adolescent or postadolescent males that this lesion, apparently rather uncommon, is most likely to be encountered.

As to location, it has already been noted that in all of our nine cases it was a long tubular bone that was affected. The lesion was found limited to part of an epiphysis or involving the epiphysis and part of the adjacent metaphysis, but never located in a metaphysis exclusively. It may be surmised that the tumor originates in an epiphysis and that if the adjacent portion of a metaphysis is affected this involvement is secondary. In one of our cases, in which the patient was observed over a period of 16 months, during which several roentgenograms were taken, the growth of the lesion could be traced as it spread into the adjacent metaphysis.

In regard to our own cases, it was found that in four the lesion was in the lower epiphysis of a femur, involving also the adjacent metaphysis in two of these. In three cases it was in the upper epiphysis of a tibia, involving the adjacent metaphysis in two again. In one case it was in the lower epiphysis and adjacent metaphysis of a tibia. In still another case it involved the tuberosities, part of the capital epiphysis and the adjacent portion of the metaphysis of a humerus.

In connection with localization, it remains to be noted that in all of the nine cases reported by Codman [3] the lesion was in the upper end of a humerus. Indeed, such references to this lesion as exist in textbooks and articles sometimes include the statement that there is predi-

lection for this region and, perhaps, even that it is found in it alone. Codman did not think it necessarily had such a restricted localization, but he did think that that localization was characteristic for it. As already indicated, our experience does not confirm this idea. There can be no doubt that when Codman was culling his cases from the Registry of Bone Sarcoma he was concentrating his attention particularly upon the shoulder region, in accordance with his special interest. However, he brings out a useful point in regard to localization in the upper end of the humerus, namely, the prominence of involvement of the tubercles * and the sparing or limited involvement of the capital epiphysis.

Roentgenographic Appearance

In roentgenograms (Figs. 1 to 6), the tumor is oval or round, and, as noted, may be restricted to part of an epiphysis or involve also part of the adjacent metaphysis. Here the lesion may be so eccentrically located as to bulge out the cortex. Those lesions which are restricted to the epiphysis are smaller, as a rule, than those involving the adjacent metaphysis as well. In neither instance does the lesion attain a very large size. In our cases, the maximum diameter usually ranged between 3 and 6 cm., though we had two cases in which it was only about 1 cm.

The lesion appears as a fuzzily rarefied and even mottled focus which tends to be delimited by a rather well defined, narrow, encircling line from the neighboring bone. It may appear somewhat trabeculated in addition, especially when viewed from certain angles. The fuzzy, mottled appearance of the tumor reflects the calcification that is going on unevenly within it. Beyond the limits of the tumor, but not necessarily evenly around the lesion, a smaller or larger area of the bone may appear densified.

Toward the joint surface, the lesion may, and frequently does, at some point, extend to the articular cartilage, and may even bulge into the joint. Thus in one of our cases in which the tumor was in the upper epiphysis of a tibia, it had expanded the intercondyloid eminence and led to deformity and protrusion of the tibial spine. In two of our cases, a tumor in a lower femoral condyle also protruded forward

* The greater tubercle of the humerus nearly always develops from an ossification center separate from that of the head proper. This separate center may appear between the 8th and 12th months and has almost always appeared by the 21st month if it is destined to develop at all. The commonest time of union of this epiphysis with that of the head is in the 7th year, but it may take place at any other time between the 4th and 12th years. Whether a center of ossification separate from that of the head develops for the lesser tubercle has not yet become clearly established, and even those who maintain that it does are not consistent as to the chronology of its appearance and of its fusion with the head.

into the joint, through the region of the intercondyloid fossa. In the case described by King,[7] an illustration showed the tumor presenting between the condyles of the femur, the tumor having its origin in the outer condyle. In connection with the question of extension of the tumor to the articular surface of the affected epiphysis, it may be well to call attention to Codman's [8] statement that the tumor does not at any point destroy the bone to the very cartilage. His statement is based upon findings (mainly roentgenographic) in respect to lesions in the upper end of the humerus and particularly those developing in the vicinity of the tubercles. Nevertheless, in the resected specimen from a lesion in this region we found that the tumor had invaded the capital epiphysis near the tubercle and actually extended to the deep surface of the articular cartilage, causing the latter to bulge over a small area and even destroying it in part.

Clinical Complaints

In our cases, the complaints had been present for 3 months to 1 year before medical aid was sought. Some of the patients gave a history of an injury—usually a fall or twist—which they connected with the condition in some way, while others gave no history of any possibly relevant injury. All related their complaints to the affected joint region, which was generally reported to be painful, more or less swollen and limited in motion. Examination usually revealed also some increase of local heat, a point of tenderness, limping when a lower extremity was concerned and even some flexion deformity, in association with local muscular atrophy. Slight hydroarthrosis, too, was discovered in some cases.

In relation to the upper end of the humerus, the lesion has become at least somewhat familiar clinically, and is likely to be correctly diagnosed even without tissue examination, by those who know it. When it is not, it is most likely to be clinically considered a chondrosarcoma or an osteogenic sarcoma. A lesion in the knee region may be misdiagnosed clinically as tuberculosis. On the other hand, if a neoplasm is considered, it is most often thought to be a giant cell tumor. Actually, the relative smallness of the lesion and its mottled, spotty appearance in the roentgenogram (aside from the youthfulness of the subjects), should promptly rule out giant cell tumor.[1]

Treatment

As noted, except in relation to the shoulder region, cases of benign chondroblastoma of bone are not yet usually diagnosed clinically. Thorough curettement of the lesion appears to be the method of choice,

and is indeed likely to have been done before the diagnosis has been established on a histologic basis. In those of our cases in which this treatment was carried out, the lesion healed without recurrence, and function was restored. The end results in these cases were the same, whether or not the thorough curettement had been followed by moderate radiation therapy. On the other hand, in a case in which the lesion was in a femur and in which merely a punch biopsy was taken and the lesion subsequently treated with heavy irradiation, a contraction of the knee resulted, requiring arthrodesis for correction. Furthermore, in this case, islands of viable tumor tissue were still recognizable in the articular shavings removed in the course of the operative correction. It is true that Codman [8] cited two cases in which healing followed treatment by irradiation alone, but the significance of these cases is not clear, since the diagnosis was based solely upon the clinical findings and not even a punch biopsy specimen was taken. Altogether, we know that *thorough* curettage alone is sufficient to cure these lesions, and we see no necessity for using irradiation, either alone or as an adjuvant to curettage. On the whole, the weight of evidence seems to be against the necessity of irradiation and is certainly against very vigorous irradiation, either with or without previous curettage.

Follow-Up

In one case still under observation, only 9 months after an incomplete curettage of the lesion, the patient is entirely free of clinical complaints and the roentgenograms show substantial recalcification of the affected area. In another case, an incomplete removal was likewise followed by evidence of rapid calcification at the site of the lesion, and the clinical complaints disappeared within 5 months, but no further information on the subsequent history is available. In three cases the patients were discharged from the outpatient department as cured 1 year after a thorough curettage, since they no longer presented any complaints clinically and there was satisfactory ossification at the site of the lesion roentgenographically. None of these three patients has returned, and for two of them it is now 12 years since their treatment at the hospital was completed. Two cases in which, after a preliminary biopsy, the lesion was treated by heavy irradiation, have been followed for 5 years, and we know that in these there has been no recurrence of the lesion, though the neighboring joint (a knee in both instances) became stiff, requiring, as noted above, an arthrodesis in one case, because of contracture. In two instances the lesion was completely resected. In one of these, in which the lesion was a small one in the condyle of a femur, resection was followed by prompt disappearance of

all clinical complaints, and a 3-year follow-up found the patient entirely well and free from evidences of recurrence. In the other case (that represented by the specimen found in the Erdheim collection) no follow-up data are available. The follow-up histories in Codman's [8] nine cases showed all the patients well 3 to 10 years after coming under treatment.

Pathology

Gross Pathology

While we were able to examine pathologic material from all our cases, only in two did we have the opportunity of seeing the lesion intact in its setting. In one instance, the tumor was a rather small one in a femoral condyle and appeared as a small, friable mass of grayish tissue. In the other case,* the tumor was in the upper end of a humerus and involved the tubercles, metaphysis and an adjacent bit of the capital epiphysis. In this specimen, the articular cartilage of the humeral head was found preserved almost throughout. Along its posterior border it showed a chain of small marginal exostoses. In addition, on the dome of the head, there was a bumpy protrusion of the cartilage, caused (as was evident from subsequent sectioning) by a subchondral hemorrhagic cystic cavity in the tumor. Most of the articular cartilage over this bump was still intact, but posteriorly the cartilage was defective over a small area and a thin layer of connective tissue filled the base of the defect.

A coronal section through the specimen (Fig. 7) showed that while laterally the tumor affected the region of the tuberosities and extended into the capital epiphysis (to the articular cartilage) and into the metaphysis, medially parts of both the capital epiphysis and the metaphysis were uninvolved. In the cut section, the tumor measured 6 cm. in its long axis and 4 cm. across. The tumor tissue was sharply delimited from the neighboring uninvolved bone and the delimiting margin was convexly lobulated. Such tumor tissue as was not cystic or hemorrhagic (as it was in fully two-thirds of the lesion) was soft but rather firm and in some places even gritty and of a gray-yellow or gray-brown color. Nowhere in the tumor could one note grossly any tissue which could indubitably be regarded as bone. However, within the tumor and quite enclosed by tumor tissue but displaced from their normal position, one could note residual islands of epiphysial cartilage plate. The cystic and hemorrhagic areas were disposed mainly about the periphery. The cysts were 1 cm. or less in diameter and were outlined by a thin

* We found the specimen (catalogued as a sarcoma) in the collection of pathologic material preserved by the late Viennese pathologist, Jacob Erdheim, now housed in the laboratory of our hospital.

wall of connective tissue. Some of the hemorrhagic areas showed cystic softening and were apparently in the process of resolution into well defined cysts.

The appearance of the tumor in this case is in harmony with that of the resected humeral specimen studied by Phemister,[6] in which the tumor proper was a soft mass of mottled brown, blue and gray tissue. In our remaining seven cases, the tumor tissue as represented by the curetted material was in part reddish gray, rather cellular and quite vascular or even hemorrhagic in appearance, and in part more grayish and gritty and flecked with yellowish calcific material.

Microscopic Pathology

One can bring out details and gain a composite histologic picture of the lesion by processing the tissue in various ways. It is worth while to study not only material which has not been decalcified or which has been decalcified in nitric acid but also material which has been decalcified in Müller's fluid. Furthermore, appropriately processed tissue should be stained not only with hematoxylin and eosin but with von Kossa's stain, van Gieson's stain and Mallory's methylene blue stain, among others.

The basic cell of the tumor, as revealed in tissue fixed in formaldehyde and stained with hematoxylin and eosin, was generally polyhedral or round and of moderate size. Its nucleus was relatively large, usually 15 to 20 μ in its greatest diameter, round or oval and more or less centrally placed. On the whole, the chromatin material of the nucleus did not appear to be distributed in any special pattern. A nucleolus, in a fairly central location, was commonly seen. Mitotic division figures ordinarily had to be searched for, but in some areas they were more numerous. The cytoplasm and the delimiting membrane of the tumor cells failed to stand out prominently in some places, though in others they were quite evident. The general run of such cells was varied by some larger uninuclear cells, a few hyperchromatic cells and an occasional small giant cell containing two or three or even a few more nuclei. Here and there were also seen larger giant cells in varying numbers with many nuclei, usually appearing in small collections. However, as has already been stated and as will be shown more fully later, these giant cells were associated mainly with secondary changes in the tumor and hence are not significant in the sense of being of fundamental importance in its cytology.

In some fields the tumor cells were closely compacted, while in adjacent fields they were more loosely packed, being separated by varying amounts of interstitial material. The latter had a semifluid, baso-

philic appearance such as to suggest edema fluid or even chondromucin. When appropriately stained, the ground substance between the more loosely packed cells was found to contain interlacing collagen fibers in some places. However, what gave character to the histologic picture in these relatively cellular areas was the presence of spotty calcification (Figs. 8, 9 and 10). In some low-power fields, as many as two or three spots of calcification could be seen, some barely perceptible and others quite large and obvious. In sections of undecalcified tissue which had been stained with hematoxylin and eosin, the calcium appeared as bluish or purple granules. The calcium granules were not only deposited between the cells but were also found in the cytoplasm of the cells, especially where the focal deposition of calcium was relatively intense. The increased calcium deposition in such areas was associated with swelling of the tumor cells.

In surveying different fields of the tumor one could thus see clumps or nodules of tumor cells with large pale nuclei and purple-staining granules in their cytoplasm, surrounded by varying amounts of calcareous, intercellular, ground substance. In some fields, also, the focal calcium incrustation resulted in the appearance of calcareous blotches. When such an area was examined under high magnification it became evident that most of the cell nuclei within it were either disintegrating or had already disappeared, and the cellular architecture was sometimes found represented merely by faint outlines of the former cell walls. Thus, where the calcification had become more intense, focal areas of necrosis appeared in the tumor tissue. The devastation of the cells within the areas of intense calcification stood out very clearly in portions of the cellular tumor which had been decalcified and then stained with hematoxylin and eosin. Neighboring areas of focal calcification and necrosis sometimes spread and fused, leaving little if any intervening viable tumor tissue. Such areas represented the yellowish calcareous bits of gross material which can be found among the curettings from a benign chondroblastoma. Altogether, when one traces the evolution of the focal calcification, one cannot doubt that the necrosis of the tumor cells is secondary to the calcification and does not provoke it (Figs. 11 and 12).

Let us consider now the fate of the calcified and necrotic areas. About the periphery of these areas was seen a process of collagenization and the appearance of what seemed to be hyalinized connective tissue. This collagenized connective tissue progressively encroached upon and replaced the necrotic and calcified débris, roughly in the manner in which new bone is deposited upon dead bone through the process of creeping replacement. In the course of this encroachment the calcified

material was confined more and more closely to the center of the area undergoing replacement and was steadily diminishing in amount. Finally, there were large patches of collagenous hyaline or chondroid tissue in place of the necrotic tissue. These areas were separated by others in which viable noncalcified polyhedral tumor cells were still present.

In the hyaline chondroid material were seen viable cells widely separated from each other within lacunae and surrounded by a ground substance which, when stained with eosin, showed an acidophilic tendency, apparently on account of its collagen content. The hyaline chondroid tissue manifested progressive organization, amounting, in some small areas, to transformation into more typically cartilaginous tissue. In rare places there was also seen deposition of calcium in the matrix of this organized chondroid material and what appeared to be islands of bone apparently formed through direct metaplasia (Figs. 13, 14 and 15).

In the description of the gross appearance of the lesion of the humerus which we had the opportunity of studying intact in its setting, it was noted that the tumor presented also areas of gross hemorrhage, softening and small cysts resulting from the organization of hemorrhage. Microscopically, it was seen that there were a good many areas of free hemorrhage in the tumor tissue and also numerous vascular spaces and even sinuses. The hemorrhages were in the vicinity of calcified necrotic areas or even within noncalcified tumor tissue. Indeed, vascular spaces and sinuses were lined throughout or in part by tumor cells proper or were bordered by wide zones of rather hyalinized connective tissue.

We come now to the multinuclear giant cells. There were indeed, as already noted, occasional small giant cells (with two or three or even several more nuclei) distributed among the polyhedral tumor cells. These giant cells were probably actual tumor giant cells formed by fusion of the smaller uninuclear cells (Fig. 8). However, about the periphery of necrotic areas undergoing hyaline and chondroid metaplasia, and especially in the vicinity of large vascular sinuses and free hemorrhage, numerous large multinuclear giant cells were seen, often in aggregates or clumps. It is the presence of many giant cells in such areas that has been the basis in the past for conceiving this lesion to be a variant of giant cell tumor. These giant cells undoubtedly represent multinuclear macrophages such as are commonly found in any skeletal lesion in the vicinity of local hemorrhage, organization, fibrosis, chondrification, or ossification (Fig. 16).

Sections of the osseous tissue beyond the advancing margin of the

tumor showed densification of the spongy trabeculae and fibrosis of the marrow. Where the tumor had approached the articular cartilage the latter was found to be thinned out and eroded or even completely destroyed. When the tumor was in the vicinity of the attachment of an articular capsule, the synovium in that region was likely to show villous hypertrophy and fibrous thickening in consequence of irritation.

Discussion

Our object is to show: (1) why the lesion in question does not represent a giant cell tumor; (2) why we interpret its basic tumor cells as cartilage germ cells; and (3) why we think the lesion should not, however, be regarded as an ordinary benign chondroma and also not as a chondrosarcoma or osteogenic sarcoma.

The question of the correctness of the common interpretation of the lesion as a giant cell tumor of bone (albeit a variant) logically brings up the question of the definition of giant cell tumor. We [1] interpret the latter as a genuine neoplasm arising from the undifferentiated supporting connective tissue of the marrow and composed of a more or less vascularized network of spindle-shaped or ovoid stromal cells, heavily, though not necessarily evenly, interspersed with multinuclear giant cells which are part of the tumor tissue itself. Furthermore, we have found that a giant cell tumor of bone shows but little collagenous differentiation of its stroma, no evidence of calcification and only rarely any evidence at all of ossification.

Certainly, benign chondroblastoma of bone does not meet these cytologic criteria. In the first place, in relatively unmodified areas of the tumor, the basic tumor cells are polyhedral in shape and closely packed or interspersed with a small amount of basophilic intercellular material which suggests edema fluid or chondromucin. All through such areas, one can note spots and patches of calcification of this interstitial material and even of the local tumor cells proper. In such cellular and relatively well preserved areas of the tumor, large multinuclear giant cells are very sparse or altogether lacking, and thus clearly do not constitute an essential element in the pattern of the lesion. Those few giant cells that are found in such areas usually have only a small number of nuclei and apparently represent multinuclear tumor cells derived by fusion of uninuclear polyhedral cells. As already noted, it is only in those areas of a benign chondroblastoma in which there has been a considerable amount of necrosis and hemorrhage, or around those areas showing chondroid organization, that one may find many giant cells. Furthermore, these giant cells show a patchy or clumped arrangement, are larger than the tumor giant cells and may be regarded as representing multinuclear macrophages.

It might be argued at this point that it is precisely because of the cytologic differences which have been noted between benign chondroblastoma and the regulation giant cell tumor that the former should be called a variant * of the latter. However, if one does so regard it, one is placing a stronger emphasis upon superficial resemblances than upon fundamental practical differences, for these two lesions are not only different cytologically but also in respect to their clinical manifestations and prognosis. Specifically, in contrast to benign chondroblastoma, the regulation giant cell tumor is generally a rather large lesion, is seen in older subjects (20 years of age or more), does not present histologic or roentgenographic evidence of calcification and is often an aggressive lesion, frequently recurring, even becoming malignant, and requiring wide excision or amputation of the affected part.

We must now answer the question of why we are interpreting the tumor specifically as a "benign chondroblastoma" and its cells as cartilage germ cells. First, it may be useful to define cartilage tissue. Various histologists are by no means in accord on this definition.[17] Discarding academic disputes, one can say that cartilage tissue in the strictest sense is tissue whose cells produce an interstitial substance or matrix which embeds these cells and contains fibers. This matrix has

* Altogether, a whole theoretical superstructure of so-called "giant cell tumor variants" has been uncritically built up, encasing other lesions also. Thus we[8] have found no evidence to support the idea, fathered by Mönckeberg,[9] developed by Konjetzny[10] and adopted also by Geschickter and Copeland,[11] that solitary bone cyst represents a healing form of giant cell tumor. Also, we cannot sustain Ewing's[4] conception of "certain benign circumscribed spindle-cell myxosarcomas with few or no giant cells" as representing a form of giant cell tumor, and we believe that most of these represent, instead, solitary expressions of the disorder which we[12] have described under the head of "fibrous dysplasia of bone." The lesion which has been held (by Kolodny,[13] for instance) to represent the "xanthic" variant of giant cell tumor is not, to our mind, a giant cell tumor either, but rather a spindle cell fibroma of bone (nonosteogenic fibroma[14]) merely having a sprinkling of giant cells, often containing hemosiderin-bearing phagocytes and sometimes showing areas having groups of lipophages. Examples of this same lesion without the presence of lipophages are also described (by Geschickter and Copeland,[15] for instance) as examples of "giant cell variants of the bone cyst in the metaphyseal ends of the long bones" (p. 268) and the "giant cell variant of osteitis fibrosa" (p. 300, Fig. 195). Furthermore, osteofibromas (osteogenic fibromas) of bone, when appearing elsewhere than in the skull and particularly the jaw bones, have also been referred to (for instance, by Geschickter and Copeland[15]) as the "spindle cell variant" of giant cell tumor (p. 339, Fig. 219). Finally, we feel that the resemblance between the so-called "brown tumor" of hyperparathyroidism and certain epulides, on the one hand, and genuine giant cell tumor, on the other hand, is merely a very superficial cytologic one, and that the pathogenetic differences between the "brown tumor" and giant cell tumor are very much more important.[16]

In summary, we point out our doubts as to the propriety of forcing into the category of giant cell tumor, albeit as variants, certain bone lesions which bear merely a superficial resemblance to it. These supposed variants have been classified as variants of giant cell tumor mainly because they contain larger or smaller numbers of multinuclear giant cells, rather than on the basis of their total histologic structure. It is not only theoretically inaccurate but practically disadvantageous to force into the category of giant cell tumor, lesions which are fundamentally different from it in histologic appearance and prognosis.

the special physical properties of firmness, elasticity and pliability, and its special chemical contents are chondroalbuminoid, chondromucoid and chondroitin-sulfuric acid, plus collagen. Furthermore, such cartilage tissue, which is mainly the so-called hyaline cartilage, tends to show basophilism of its ground substance, and (as a feature preliminary to its degeneration and replacement) swelling of the cells and calcification of its ground substance.

If we project these features of cartilage tissue against the tissue of the tumor we are discussing, we find indications which favor an interpretation of this tumor as having much of the nature of cartilage about it. Thus, the basic cells of the tumor produce a basophilic ground substance containing collagen fibers which surround and embed the cells. Wherever the tumor tissue is calcifying, its cells swell and become separated by even more of the ground substance, which becomes impregnated with calcium. The only way in which the tissue of this tumor differs from true hyaline cartilage is that swelling of its cells and calcification of its matrix are not precursors to replacement by osseous tissue through the process of endochondral ossification. However, a hyaline chondroid tissue does replace the calcified and necrotic tumor tissue and may even undergo spotty ossification by the process of direct metaplasia. Thus there is a very definite aura of cartilage about the whole tumor.

Nevertheless, it would be incorrect to interpret this tumor simply as a chondroma of bone. If it were an ordinary chondroma, even a modified one, its full-fledged cartilaginous nature would be obvious both grossly and microscopically. Central chondromas of bone are composed of facets or mosaics of mature, well differentiated cartilage cells, between which there is likely to be, at least in many parts of the lesion, a richly hyaline matrix. Even in areas in which the intercellular matrix is meager or has undergone myxoid or mucoid degeneration, the cells and the tissue as a whole still retain the character of actual cartilage. None of this is true in regard to benign chondroblastoma. Hence, the tumor cells of this lesion, which do not mature into full-fledged cartilage cells, should, in our opinion, be interpreted as chondroblasts, and the tumor itself as a chondroblastoma, the word "benign" being prefixed to indicate that the lesion is not a malignant one, in spite of its richly cellular character.

To refute the other idea—that the lesion is a chondrogenic or osteogenic sarcoma—we would need only to refer to the uniformity with which the lesion has been found benign by those who have studied it closely. In addition, a chondrosarcoma not only develops out of full-fledged cartilage but shows cellular, and particularly nuclear, atypism

of this cartilage, such as one does not see in chondroblastoma. Specifically, a chondrosarcoma shows numerous binuclear cartilage cells with plump nuclei, large or giant cartilage cells with single or multiple nuclei, and, if it is full-blown, more than a sprinkling of mitotic division figures. Cytologically, there should also be no confusion with osteogenic sarcoma. The latter develops out of bone-forming mesenchyme, and, in contrast to benign chondroblastoma, not only may produce a considerable amount of clear-cut cartilage by proliferation, but always lays down some tumor osteoid tissue and bone directly between the tumor cells proper.

Summary and Conclusions

The lesion which we have described and called benign chondroblastoma of bone has usually been conceived in the past as a variant of giant cell tumor (calcifying or chondromatous) and sometimes even as a malignant tumor of bone and specifically a chondrosarcoma or an osteogenic sarcoma. The tumor starts its development in an epiphysis, usually of some long bone. It may extend to the articular surface of the epiphysis and even into the metaphysis, but nevertheless rarely attains a size of more than 3 to 5 cm. in its largest diameter. We did not find that the lesion exhibits a predilection for the upper end of the humerus, although some previous workers have thought that it did. It does seem to occur particularly in males, and the patients are almost always adolescents or postadolescents at the time when they seek medical care for the condition. When the roentgenogram is considered in the light of the pathology of the lesion, there is much about it to help make the diagnosis on a clinical basis.

The basic tumor cell of the lesion is polyhedral or round and of moderate size, with a fairly large nucleus. The tumor cells may be closely packed or more loosely agglomerated, but what is very distinctive of the pathology is the presence of focal areas of calcification of the cellular tumor tissue. Wherever the calcification becomes intense, the tumor cells swell and undergo necrosis. The necrotic tumor tissue comes to be replaced by hyaline chondroid tissue which subsequently may show spots of ossification. There may be areas of hemorrhage, and one may see large vascular sinuses bordered by viable tumor tissue, necrotic tumor tissue, or hyaline chondroid material which has replaced the necrotic tumor tissue. Clumps of large multinuclear giant cells may be seen in the areas of hemorrhage, about the vascular sinuses and even in the hyaline chondroid tissue.

The lesion is entirely benign and heals without recurrence after thorough curettage, even without supplementary radiation. We think

it is not proper to regard this lesion as a variant of giant cell tumor and hold the tumor cells to be chondroblasts and the tumor a benign chondroblastoma.

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DESCRIPTION OF PLATES

PLATE 150

FIG. 1. Roentgenograph showing a lesion 1.5 cm. in diameter, located in, and limited to, the lower epiphysis of a femur and, specifically, to the external condyle. Radio-opacity may be seen, indicating sclerosis of the bone beyond the limits of the lesion. The patient was a boy of 13 years who complained of swelling, tenderness and weakness of the knee, walked with a slight limp and presented some local heat and muscle atrophy. There was no antecedent history of injury.

FIG. 2. Roentgenograph showing a lesion 4.5 cm. in its greatest diameter, located in the lower epiphysis of a femur, specifically in the intercondylar region, and also involving the metaphysis. There is a radio-opaque line more or less outlining the lesion. The latter casts a rather vague, fuzzy shadow which, however, yields some suggestion of trabeculation. The patient was a boy of 15 years who, for 1 year, had been having mild difficulties relating to the knee. There was a history of an injury (fall) 2 years previously, but the relevance of this is not clear.

FIG. 3. Roentgenograph showing a lesion 3 cm. in its greatest diameter, located in the upper epiphysis of a tibia and extending from the epiphysial cartilage plate to the intercondyloid eminence, where it has actually perforated into the joint. There is a fuzzy, mottled shadow cast by the lesion, and the surrounding bone is radio-opaque. The patient was a boy of 14 years who complained of difficulty with his knee (stiffness, swelling, limp) for 3 months before admission to the hospital. His history also included the statement that he fell from a bicycle about 1 month before admission, but this fall could not be clearly related to the present bone disease.

FIG. 4. Roentgenograph showing a lesion 5 cm. in its greatest diameter, located in the proximal epiphysis of the tibia (involving parts of both condyles) and also extending into the adjacent metaphysis. The lesion appears to be vaguely mottled, with a delimiting line about it. The patient was a boy of 17 years who had been complaining for about 6 months of slight swelling of the knee (especially on walking), some local heat and tenderness, and some limitation of flexion. His history included the statement that 7 months prior to admission he had tripped and injured his knee.

FIG. 5. Roentgenograph showing a lesion 4 cm. in its greatest diameter, located in the lower epiphysis and metaphysis of a tibia. In this view the x-ray shadow cast by the lesion is rather vague. The patient was a boy of 15 years who complained mainly of some pain in the ankle, of several months' duration.

FIG. 6. Lateral view of the lesion shown in Figure 5, indicating more precisely that the lesion is situated posteriorly and has bulged out the cortex of the tibia. In this view the lesion shows considerable trabeculation.

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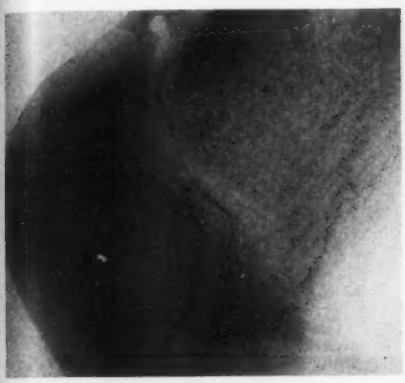

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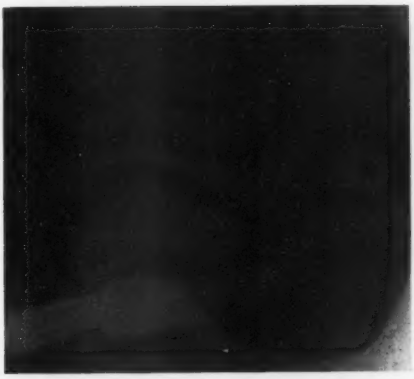

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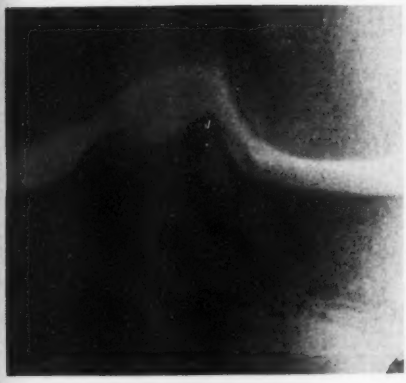

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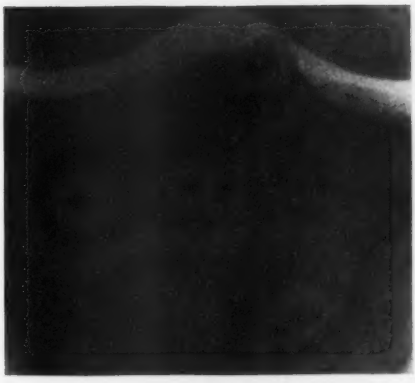

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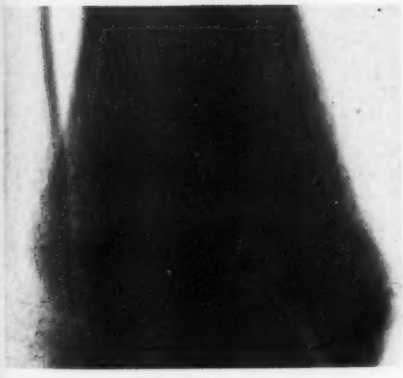

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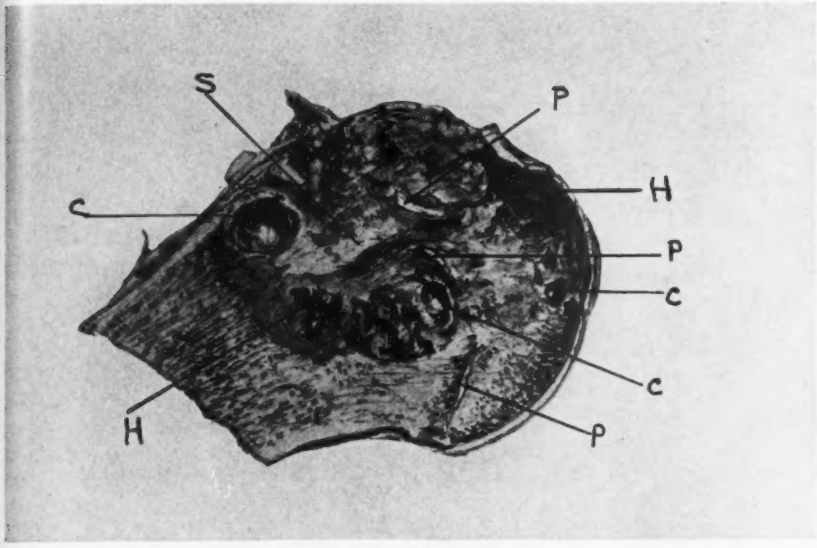
Jaffe and Lichtenstein

Benign Chondroblastoma of Bone

PLATE 151

FIG. 7. Drawing (actual size) of coronally cut upper end of a humerus showing a benign chondroblastoma involving the tuberosity and adjacent portions of the capital epiphysis and metaphysis. The specimen came from a boy of 16 years and was obtained by surgical resection done on the assumption that the lesion was a sarcoma. It may be seen that the tumor extends to the articular cartilage of the head and that this cartilage actually bulges a little where the head is involved. The tumor tissue proper was a whitish gray-brown in color. C = cysts, H = areas of hemorrhage, P = bits of epiphysial cartilage plate and S = a large area of chondroid scar tissue.

FIG. 8. Photomicrograph showing the general cytologic pattern in a cellular and relatively unmodified area of a lesion. The stippling toward the left of the picture represents beginning calcification between some of the cells. Toward the center there is beginning swelling of the cells and a small multinuclear giant cell. × 450.



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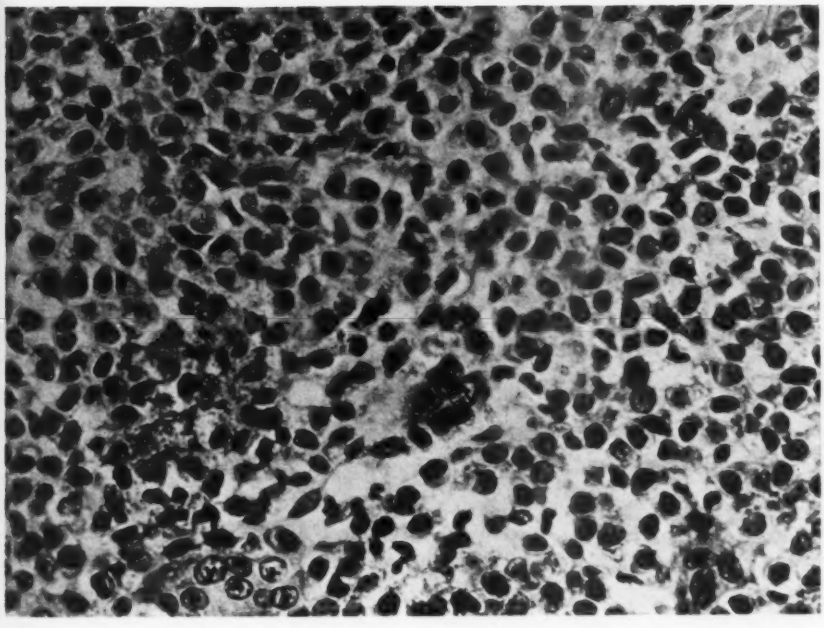

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Jaffe and Lichtenstein Benign Chondroblastoma of Bone

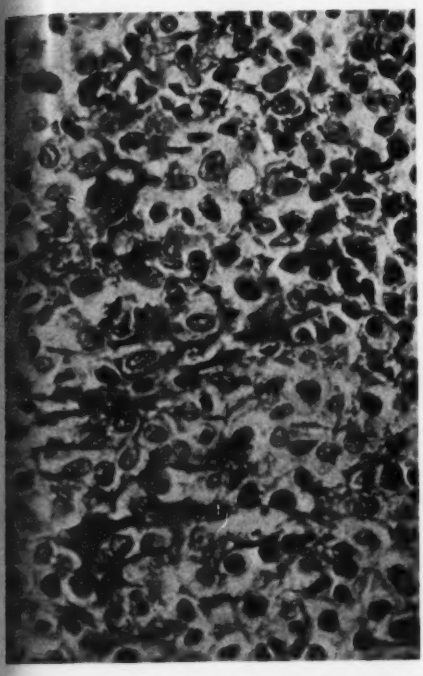
PLATE 152

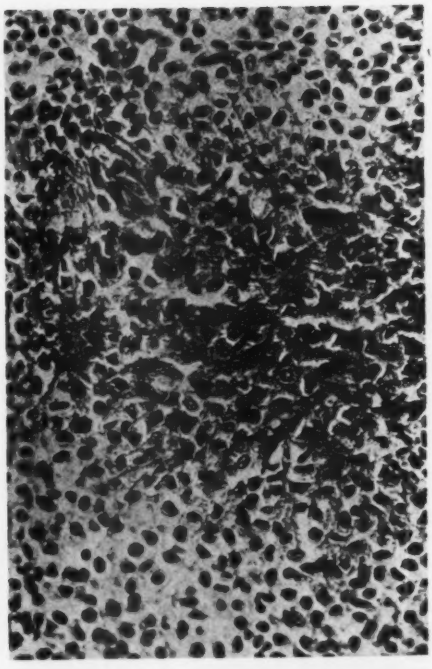
FIG. 9. Photomicrograph showing the general cytologic pattern in a cellular area in which the deposition of intercellular material and the process of calcification have advanced farther than in Figure 8. × 450.

FIG. 10. Photomicrograph showing a nidus of even more densely calcified tumor tissue surrounded by tumor tissue not yet so modified. The tissue from which the slide was prepared had not been treated with nitric acid before being embedded. The grittiness of such a calcareous area (and, *a fortiori*, of a more calcareous one) is likely to result in fragmentation when the slides are prepared from paraffin-embedded tissue, so that satisfactory photographs of intensely calcified areas are difficult to obtain. × 230.

FIG. 11. Photomicrograph showing an area in which the calcification had originally been more intense than that shown in Figure 10, but decalcified with nitric acid before embedding. Pale shadows of necrotic cells may be seen where the calcification has been particularly intense. × 230.

FIG. 12. Photomicrograph showing intermingled viable and necrotic (and previously heavily calcified) tumor tissue with areas of cystic softening. The tissue from which this slide was prepared had been treated with nitric acid. × 100.





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Benign Chondroblastoma of Bone

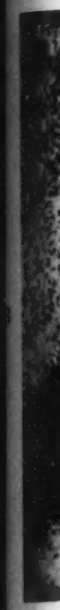
PLATE 153

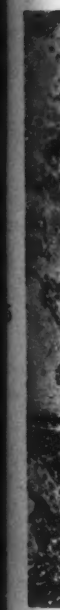
FIG. 13. Photomicrograph of tissue which, after being decalcified in Müller's fluid, has been stained with hematoxylin and eosin. The dark blotches represent areas of necrotic and still calcareous tumor tissue, between and about which collagenization and chondroid metaplasia has been taking place. × 53.

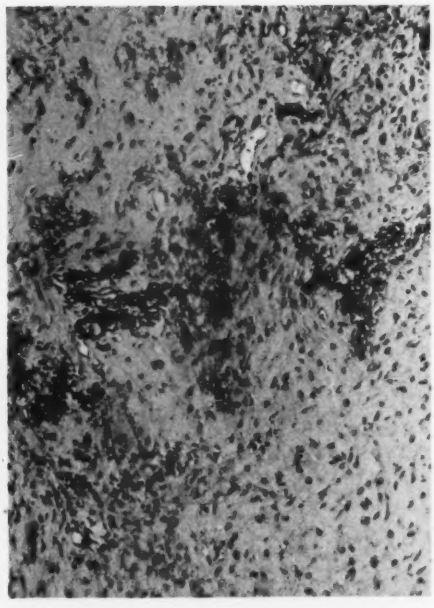
FIG. 14. Photomicrograph showing further progress in the process of collagenization and chondral replacement of a necrotic area. Some residual necrotic and calcified tissue is seen, surrounded by chondral material. × 72.

FIG. 15. Photomicrograph showing chondroid areas intermingled with cellular tumor areas. Here and there in the chondroid tissue secondary calcification is apparent. In some lesions, the calcifying chondroid material may undergo spotty osseous metaplasia. × 72.

FIG. 16. Photomicrograph showing an area of cellular tumor in which there has been blood leakage, the hemorrhagic area being outlined by multinuclear giant cells. × 220.







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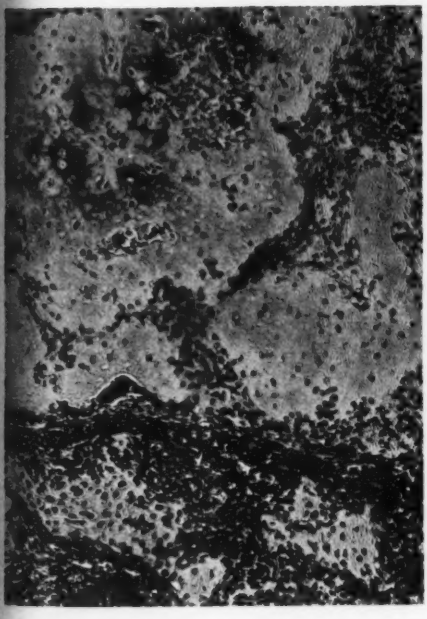

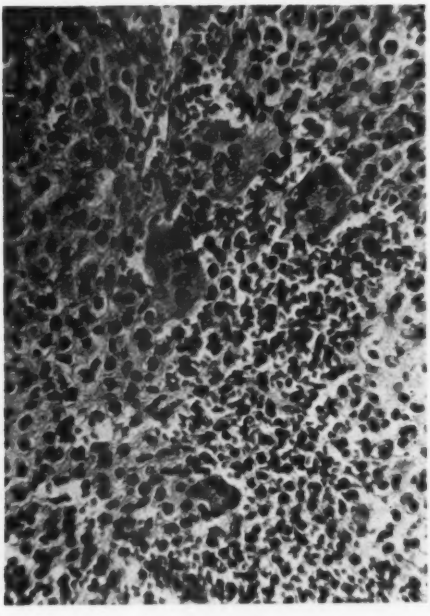

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Jaffe and Lichtenstein Benign Chondroblastoma of Bone

CARCINOID TUMORS OF THE RECTUM DERIVED FROM ERSPAMER'S PRE-ENTEROCHROME CELLS *

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Although a great deal has been written about carcinoid tumors, there exists very little information about their occurrence in the large bowel. In the Laboratory of Surgical Pathology of Columbia University there are records of four cases of reputed carcinoid of the rectum, and in the neighboring Laboratory of Pathology are two other rectal cases found among 13,809 routine autopsies. There are no examples of carcinoid tumors in the cecum or colon. Table I shows the distribution of all the cases involving the large bowel known to me. A study of the Columbia University cases has shown that the carcinoid tumor in the rectum sometimes displays certain morphological features which differ from the commoner carcinoids found in the appendix and small intestine. These variations seem worth recording because it is not easy to recognize these tumors as carcinoids unless one is familiar with them. I had been uncertain about the nature of case 1 for 9 years, and no one to whom I submitted it was able to help me. Finally, a review of the more recent histological investigations of the basigranular cells of the gastrointestinal tract afforded a reasonable explanation of the vagaries shown by the tumors reported in this group and made it possible to trace such seemingly dissimilar neoplasms, as are described in cases 1 and 2, to a common ancestor.

REPORTS OF CASES

Case 1

C. L., a married American negress, 19 years of age, came to the Proctology Clinic of the Presbyterian Hospital in January, 1932, suffering with bleeding from the rectum and increasing constipation of 1 year's duration. Examination showed a rounded polypoid mass, 2 cm. in diameter, situated anteriorly in the lower rectal segment and attached to the first valve, about 5 cm. from the anus on the left side. The mass was movable, without infiltration at the base. Biopsy was done on January 8, 1932, and on January 26, 1932, a wedge-shaped segment of the rectal wall, including the tumor, was excised. In September of the same year a simple adenomatous polyp was removed from near the site of excision. The patient has been followed continuously since and remains without any evidence of local disease. She was last seen on May 21, 1941. She had enlarged right cervical nodes believed to be due to tuberculosis and enlarged tonsils. Otherwise, physical examination was negative.

Gross Examination. The specimen consisted of a segment of rectal wall about 2 cm. long and a submucosal tumor, 18 by 17 by 15 mm.

* Received for publication, March 2, 1942.

(Fig. 1). The tumor was covered with elevated mucosa to which it was attached in places. An intact collar of mucosa varying from 2 to 7 mm. in width surrounded it and deep to it were the intact muscle coats of the rectum. The cut surface of the tumor was firm and appeared pearl

TABLE I
Carcinoid Tumors of the Large Bowel

Author	Anatomical distribution		
	Cecum	Colon	Rectum
Ariel	0	1	0
Brunschwig	0	0	1
Corten	1*	0	0
Feyrter	0	5	0
Humphreys	0	0	1
Kaufmann	0	1	0
Koch	0	0	1*
Mallory	0	0	1
Martin	0	1	0
Mayo and Wilson	1*	0	0
Raiford	1*	1*	0
Saltykow	0	1	1
Siburg	0	0	1*
Stout	0	0	6
Wyatt	1*	0	0
Total	4	10	12

* Indicates malignant carcinoids.

white, faintly tinged with yellow, and mottled with red due to extravasated blood. The specimen was fixed in Bouin's fluid.

Microscopical Examination. The tumor consisted of large, solidly packed masses of cells loosely held together by a delicate supportive framework of widely spaced reticulin fibers bearing infrequent blood vessels (Fig. 1). This was easily seen with the Laidlaw silver reticulin stain but was very inconspicuous with Masson's trichrome stain and still more so after hematoxylin and eosin. At its periphery the tumor cells broke up into more definite strands. In places they lay just beneath the muscularis mucosae and sometimes passed through it to appear in the stroma surrounding the crypts of Lieberkühn. In three different places the crypts had disappeared and the tumor had reached the surface. The tumor cells nowhere appeared in direct continuity with the cells of the crypts. Extravasated blood was found in several parts of the tumor and many tumor cells showed pyknosis but there was no extensive necrosis. In the depths, the tumor remained within the confines of the submucosa and at no point penetrated the inner muscle coat.

The individual tumor cells were large, irregularly rounded and occasionally columnar or prismatic, with poorly defined cell membranes in

most parts, so that it was difficult to determine the outlines of most of them. Where this was seen the nucleus generally occupied an eccentric position. The nucleus was quite large, with sharply defined chromatin (Fig. 2). No mitotic figures were observed. The cytoplasm was very finely granular and in general stained a neutral tint. The most striking and characteristic feature was the presence of slightly larger fuchsinophil granules found in the cytoplasm at one side of the nucleus in a very considerable number of the cells. These granules were best seen when Masson's aniline blue-ponceau-acid fuchsin stain was used. They were not differentially stained with eosin and after 96 hours in ammoniacal silver nitrate they showed only the very faintest browning. In this same preparation the granules of the basigranular cells of the crypts of Lieberkühn were jet-black. In the section stained by Masson's trichrome method there were a number of basigranular cells in the crypts of Lieberkühn with fuchsinophil granules (Fig. 2), but not nearly as many as had silver blackened granules after treatment with ammoniacal silver nitrate.

The degenerated cells generally showed pyknotic nuclei and had cytoplasmic vacuoles which were reddened with scharlach R. Lipoid-filled vacuoles were occasionally found in well preserved cells. The tumor cells nowhere formed acini, rosettes or pseudorosettes. They did not secrete mucin or colloid.

Case 2

L. S. was a British West Indies negress, 41 years of age. Her trouble began 4 months before admission with diarrhea and pain in the left lower quadrant of 5 days' duration followed by constipation, anorexia, nausea, loss of 9 lbs. in weight and persistence of the pain. Proctological examination showed a 5 mm. sessile tumor situated 5 cm. from the mucocutaneous junction on the left anterior wall of the rectum. On October 4, 1938, the tumor was locally excised. It was later found that her symptoms were due to regional enteritis and on April 3, 1939, an ileocolectomy was done at another hospital. Examination did not show any other carcinoid tumors. She was known to be alive on October 19, 1940.

Gross Examination. The specimen was received in Bouin's fluid. It consisted of an intramucosal, seemingly circumscribed, tumor nodule, measuring 7 by 4 mm. and surrounded by a thin collar of mucosa between 1 and 2 mm. wide.

Microscopical Examination. The tumor lay chiefly in the submucosa but extended through the muscularis mucosae into the overlying mucosa which was much thinned and atrophic. The tumor had a very striking appearance, being composed of cords of slender columnar and prismatic cells so arranged that the long axis of the cells was at right angles to the long axis of the cord. The cells were in a single layer and the cords were bent and curled so as to resemble heaps of ribbon carelessly coiled

at haphazard (Fig. 3). The nuclei were elongated to fit the narrow shape of the cells and were generally oriented toward one pole. The chromatin was arranged as in the nuclei of case 1, and did not have the large prominent nuclei characterizing the mucous cells of the crypts of Lieberkühn. Slender strands of dense fibrous tissue separated the cords, and thicker strands separated groups of cords. In one place a tumor cord united with cells forming a crypt of Lieberkühn in such a way that the bottom of the crypt was formed of tumor cells (Fig. 4). A search through 42 sections showed only a few mitotic figures. These were found in tumor cells in the mucosa close to the goblet cells of the crypts in which mitosis was very active. The cytoplasm contained a few diffusely scattered granules which were faintly acidophilic with eosin and fuchsin but which were never strongly acidophilic as in case 1, and were not blackened by ammoniacal silver nitrate even after 96 hours. In this same preparation the granules of the basigranular cells in the crypts of Lieberkühn were clearly defined and a dark brown. No attempt to form rosettes or pseudorosettes was observed nor was any mucin or colloid formed. A few empty, basally situated intracellular vacuoles were seen. A scharlach R stain showed that these contained lipoid. No tubules were formed, except in a few instances where cords of tumor cells were found in the mucosa. The tumor seemingly had not infiltrated deeply, as an uninvolved layer of submucosa lay deep to it.

Case 3

L. H. was an American negress, 34 years of age. She complained of soreness and anal protrusion after stool, which began 4 years before admission, and had recently grown worse, with bleeding. By proctological examination, hemorrhoids were found as well as a firm, sessile, rounded mass about 1 cm. in diameter on the anterior rectal wall about 5 cm. above the anal margin. It had a mottled, yellow appearance. It was excised with a surrounding collar of mucosa on November 7, 1941. When histological examination seemed to show that excision was incomplete, a second operation was done 11 days later and the rectal wall in the region of the scar was excised through an abdominal approach. No tumor was found in this specimen. The patient was well when examined 2 months later.

Gross Examination. The tumor measured 11 by 8 mm. and had some ragged tags of mucosa attached to it. It had a yellowish gray tinge and was sharply defined. The cut surface was also grayish. It was fixed in Zenker's fluid with 5 per cent glacial acetic acid added.

Microscopical Examination. This tumor resembled that of case 2, with certain differences (Fig. 3). It was composed of strands and ribbons of columnar and prismatic cells arranged in festoons, but the cells were shorter and slightly plumper so that the nuclei tended to be rounded. The cells tended to shrink away from the supporting fibrous stroma so that they appeared to lie in spaces. As shown in Figure 3,

the twisted cords of columnar cells sometimes united abruptly with solid masses of polygonal tumor cells which resemble those of ordinary carcinoids. No tumor cell mitoses were seen, although there were many in the mucous cells of the crypts. The tumor cell cytoplasm contained many tiny granules but these were not fuchsinophil and they were unaffected by long immersion in ammoniacal silver nitrate. In the crypts of the overlying mucosa were many basigranular cells which were strongly fuchsinophil but only a few cells had granules darkened by ammoniacal silver nitrate. The tumor lay chiefly in the submucosa but separated the fibers of the muscularis mucosae, and strands of tumor cells were found between the crypts. Some of these had been destroyed and in others the deeper portion of the crypt was composed of tumor cells. The sections did not show the relation of the deep surface of the tumor to the rectal wall. No tubules, rosettes, or pseudorosettes were formed. No mucin or colloid had been secreted. Some cells contained empty vacuoles. Lipoid stains were not done.

Case 4

Mrs. S. L. was an American Jewess, 38 years of age. Two months before operation proctoscopic examination showed a small, firm nodule on the anterior wall of the rectal ampulla. In 2 months' time this increased to a diameter of 5 mm. There was rectal bleeding 2 days before operation, ascribed to hemorrhoids which were present. A barium enema showed no other abnormalities of the large bowel. The nodule was excised with a fulgurating snare on November 13, 1939, and the base was cauterized with the actual cautery. There has been no evidence of recurrence since that time. (The case has been made available by Dr. Alvin O. Severance of San Antonio, Texas, who recognized it as a carcinoid tumor. Several other pathologists, including two with national reputations whose experience with tumors is very large, had called it carcinoma.)

Gross Examination. When received by Dr. Severance the specimen measured 6 by 7 by 4 mm. and was covered on one surface with apparently intact mucosa. The nodule was fixed in formaldehyde.

Microscopical Examination. Like the three preceding cases this tumor lay in the submucosa and extended between the layers of the muscularis mucosae where its strands were found in some places replacing the crypts, elsewhere between the crypts and in one instance united with the cells of a single crypt. The strands of tumor cells were predominantly columnar or prismatic, with rounded, ovate, or cylindrical nuclei showing no mitoses and situated usually toward one pole. The cytoplasm was partly granular and partly amorphous but the granules were not fuchsinophil nor were they affected by ammoniacal silver nitrate after immersion for 72 hours. In several areas, however, instead of ribbons of coiled columnar cells arranged in festoons, there were polygonal cells crowded together in large solid masses as in case 1.

In the overlying crypts a few basigranular cells with fuchsinophil granules could be identified with difficulty, but none with browned or blackened granules were noted after silvering. No tubules, rosettes, or pseudorosettes had been formed and no mucin or colloid was secreted. Fat stains were not done.

Case 5 *

J. K. was a white American female, 42 years of age, who died from infection following perforation of the esophagus.

Gross Examination. At autopsy a firm, white, oval plaque, 7 mm. in diameter, was found in the submucosa of the anterior rectal wall. The specimen was fixed in Zenker's fluid.

Microscopical Examination. Because of postmortem changes, examination was not satisfactory. Like the other tumors, this was limited to the submucosa and infiltrated the mucosa. It was made up of widely separated strands of polygonal cells, smaller than those of the four preceding tumors and more nearly like those in the carcinoids of the appendix and small intestine. The cytoplasm contained granules which were not affected by fuchsin or ammoniacal silver nitrate. No tubules, rosettes, or pseudorosettes were formed and no mucin or colloid was seen. Fat stains were not done. In spite of postmortem changes in the epithelia of the crypts, basigranular cells with fuchsinophil granules were seen. They were unaffected by silver nitrate.

Case 6

J. B. was a colored American male, 43 years of age, who died following the attempted removal of a leiomyosarcoma of the stomach.

Gross Examination. At autopsy there was found an elevated, yellowish, slightly flattened, freely movable, firm nodule in the mucosa and submucosa of the rectum just above the anus. The specimen was fixed in Zenker's fluid.

Microscopical Examination. This tumor also lay in the submucosa, separated the fibers of the muscularis mucosae and ramified in the mucosa where the crypts had been eroded and in some places destroyed by it. It did not extend beneath the submucosa. Like case 3, it was made up chiefly of festoons of short cylindrical cells with hardly any tendency to form polygonal cells, which were seen in only a few places. No tubules, rosettes, or pseudorosettes were found. No special stains were done.

Discussion

Masson's (1928) description of the carcinoids of the appendix and small intestine stated that they consist chiefly of cords of round or

* Cases 5 and 6 come from the Laboratory of Pathology of Columbia University and are reported by permission of Dr. James W. Jobling, Director.

polygonal cells. Occasionally there are palisade cells next to the connective tissue outlining a cell column and rarely there are columnar and prismatic cells grouped around a cavity or vesicle which may contain a tiny albuminous droplet of colloid aspect. This description is in accord with the appearance of the appendiceal and ileal carcinoids in this laboratory, but it does not fit the appearance either of case 1, with its huge solid masses of polygonal cells, or the ribbonlike festoons of columnar and prismatic cells seen in cases 2, 3, 4 and 6. Such festoons are occasionally found in pancreatic islet adenomas as illustrated by Laidlaw (1938). Polygonal cells are present in these four tumors but they are infrequent and the striking appearance of the festoons makes it easy to overlook them. Like ordinary carcinoids, these tumors have cells with lipoid-filled vacuoles, but none shows the formation of vesicles or rosettes and none secretes albuminous material. All of them involve the mucosa and in cases 2, 3 and 4, tumor cells are found in contact with and forming part of crypts. Occasionally in the mucosa, tumor cells surround open spaces, but this arrangement is never found elsewhere. Whether the involvement of the crypts is primary or secondary is not apparent.

The reactions of the intracellular granules is also at variance with those of appendiceal and ileal carcinoids. Silvering was done in all but case 6. Case 5 will not be considered, both because it was an autopsy case and also because the technical procedure was not carried out in this laboratory. Cases 1 and 2 were fixed in Bouin's fluid, case 3 in Zenker's fluid with 5 per cent glacial acetic acid added, and case 4 in formaldehyde. In every case, Fontana's ammoniacal silver nitrate was used according to Masson's formula. In the first three cases the same preparation showed blackening or browning of the granules of the basigranular cells in the crypts but had no effect on the tumor cell granules of cases 2, 3 and 4, and caused only the very faintest browning in case 1. In case 1, the intracellular granules were strongly fuchsinophil, in case 2 they were weakly fuchsinophil, while in the other cases they did not seem to be selectively different from the rest of the cytoplasmic content. In all of the five cases, Masson's aniline blue-ponceau-acid fuchsin reddened the granules of the basigranular cells of the crypts.

At this point one naturally inquires: Are these unusual formations really carcinoid tumors derived from the basigranular cells of the mucosal crypts or are they some other kind of tumor, such as, perhaps, the islet cell adenoma to which they have certain resemblances? A heterotopia of islet cells in the rectum has not been described so far as I am aware. Therefore, unless the basigranular cells themselves are islet cells, one does not need to give this explanation serious consideration.

No case of tumor of the large bowel has given symptoms of insulin secretion: no one, including myself, has been able to demonstrate the specific staining reactions of islet cell granules in carcinoid tumors, nor, on the other hand, will islet cell granules blacken with ammoniacal silver nitrate. Therefore it seems safe to conclude that, in spite of a morphological resemblance, neither the basigranular cells nor carcinoid tumors are derived from islet cells. There remains for consideration the relationship of these rectal tumors to the basigranular cells. In order to answer this question I reviewed the information extant pertaining to these well known but mysterious cells.

In addition to Masson, there are a number of histologists and pathologists who have devoted much time and thought to the investigation of these cells. Among these should be noted especially Clara, Erspamer, Feyrter, Jacobson, Patzelt and Popoff. The work up to 1936 is extensively reviewed by Patzelt in Möllendorff's Handbuch. For the purposes of identification and of limiting these remarks to the large bowel and rectum, it may be noted that the basigranular cells are scattered at intervals among the mucus-secreting cells of the crypts of Lieberkühn. They are usually pyramidal cells with their bases resting upon the basement membrane and their apices usually not reaching as far as the lumina of the crypts. These cells have nuclei elevated above the basally situated nuclei of their neighboring cells and in the basal cytoplasm are found a considerable number of fine granules (Fig. 2). These cells have been called by a variety of terms, such as yellow cells, argentaffin or chromo-argentaffin; chromophil, chromaffin or enterochromaffin, and basigranular cells, and by the names of Nicolas, Kultschitzky, Schmidt and Ciaccio. Of all these the purely descriptive term "basigranular" seems preferable because it is neither a proper name nor a questionable attribute. Clara (1934) has shown that these cells are derived in the human embryo from undifferentiated intestinal epithelium, and Popoff (1939) believed that they continue to be formed during adult life from the mucus-secreting cells. There is some indirect support for this viewpoint in the observations of Cordier (1924) and Siburg (1929), both of whom described carcinoid tumors in which mucus-secreting cells were associated with the granular cells.

The granules have naturally excited a great deal of interest and speculation. Erspamer (1939) designated the cells as enterochromaffin and recognized four variants. The typical enterochromaffin cells have granules containing a chemical substance which he called enteramin. This makes them give a positive diazo reaction, become blackened by ammoniacal silver nitrate and show fluorescence in ultraviolet light. These typical cells are preceded by two different earlier phases. In the

first, which he called clear nonargentophil pre-enterochrome cells, there are no argentophil or argentaffin granules at all. The second stage he called pre-enterochrome acidophil argentophil cells. These contain acidophil granules which can take up silver, but as they contain no enteramin they do not reduce it and become blackened nor do they give the diazo reaction nor fluoresce. Finally, he recognized what he called empty enterochrome cells which have granules that have been emptied of enteramin. Vialli and Erspamer (1933) supposed that the specific substance in the granules (enteramin) is probably ortho-diphenol that has an unknown and perhaps not very complex side chain with a free area in the ortho-position. Jacobson (1939) found that the granules contain both a pterine and a carbohydrate.

Vialli and Erspamer first noted the acidophil pre-enterochrome cells in the large bowel of the dog and cat. It seems to be true that both types are found there also in humans.

The speculations regarding the function of the basigranular cells are interesting chiefly because they are so much at variance with each other. Masson and Berger suggested that the substance secreted by the cells is emptied directly into nerves; Erös (1933) and Jacobson (1939) believed that the secretion plays a part in erythropoiesis; Popoff (1939) thought that the granular cells have no definite function at all, but are simply stages in the rejuvenation of functionally exhausted and refractory mucous cells. Reference to Patzelt's review shows that other ideas have also been expressed such as: That the granules are an exocrine secretion, that they have a resorptive function, that they are concerned with digestion, that they are related to suprarenal medullary cells, to the oxyphil cells of the hypophysis and to the islet cells of the pancreas. It will suffice to say that none of these hypotheses stands as proved. The nature and meaning of the basigranular cells of the gastrointestinal tract remain unknown.

However, the investigations which have been carried out on these cells are of great aid in interpreting the appearance of this present group of tumors of the rectum. If the basigranular cells are formed from the goblet cells and only secondarily assume their pyramidal or wedge shape and acquire their granules, it is not so difficult to explain the variants seen in the tumor morphology. The cords or ribbons of columnar and prismatic cells represented in Figures 2 and 3 may be homologues of the basigranular cells in an earlier phase of metamorphosis, while the polygonal cells more nearly approximate the appearance of these cells as they appear in the crypts (Fig. 2). If we may believe the work of Clara and of Erspamer that the granules of the basigranular cells are ordinarily associated with a material which

enables them to blacken ammoniacal silver nitrate, while in some instances, particularly in the large bowel, the granules may lack this substance and so be unable to blacken ammoniacal silver nitrate, an adequate explanation is furnished for the observation that in cases 1, 2 and 3 basigranular cells of the rectal mucosa were darkened by silver, while the granules in the tumor cells of the same preparations were not. It is necessary to be specific about fixatives and the preparation of silver used because, as Hamperl (1932) has pointed out, whether or not silver will blacken granules depends upon these two factors. Some silver preparations, such as that of Gros-Schultze,* will blacken not only melanin and intestinal basigranular cells but also islet cells, suprarenal medulla cells and anterior hypophysis cells. In order to complete this rather complicated picture, the reaction with ammoniacal silver nitrate also depends upon the presence or absence of enteramin in the granules.

Information regarding other carcinoid tumors of the large bowel is exceedingly scanty. The reports indicate that ammoniacal silver nitrate was tried in only seven cases. It succeeded in four (Brunschwig; Wyatt; Raiford, 2 cases) and failed in three (Feyrter; Martin; Siburg). It was impossible to learn whether the basigranular cells of the overlying crypts were blackened in these last three cases.

It would seem, then, that the festooning ribbons of columnar cells represent the reproduction on a large scale of the basigranular cells in an early phase of differentiation when they approximate the shape of columnar crypt cells. The failure of the tumor cell granules to blacken ammoniacal silver nitrate after proper fixation and in the same preparations in which the granules in the basigranular cells in the crypts were blackened can be explained if one supposes that the latter contained enteramin while the tumor cell granules lacked it. If one accepts this, these tumors can be regarded as carcinoids composed of Erspamer's pre-enterochrome cells.

Summary

Six probable carcinoid tumors of the rectum are described. Five of them differ cytologically from the common carcinoids found in the appendix and ileum. It is suggested that these are carcinoid tumors composed of Erspamer's pre-enterochrome cells.

* The Gros-Schultze Silver Method for Axis Cylinders and Nerve Terminals. In: Mallory, F. B. Pathological Technique. W. B. Saunders Co., Philadelphia and London, 1938, pp. 227–228.

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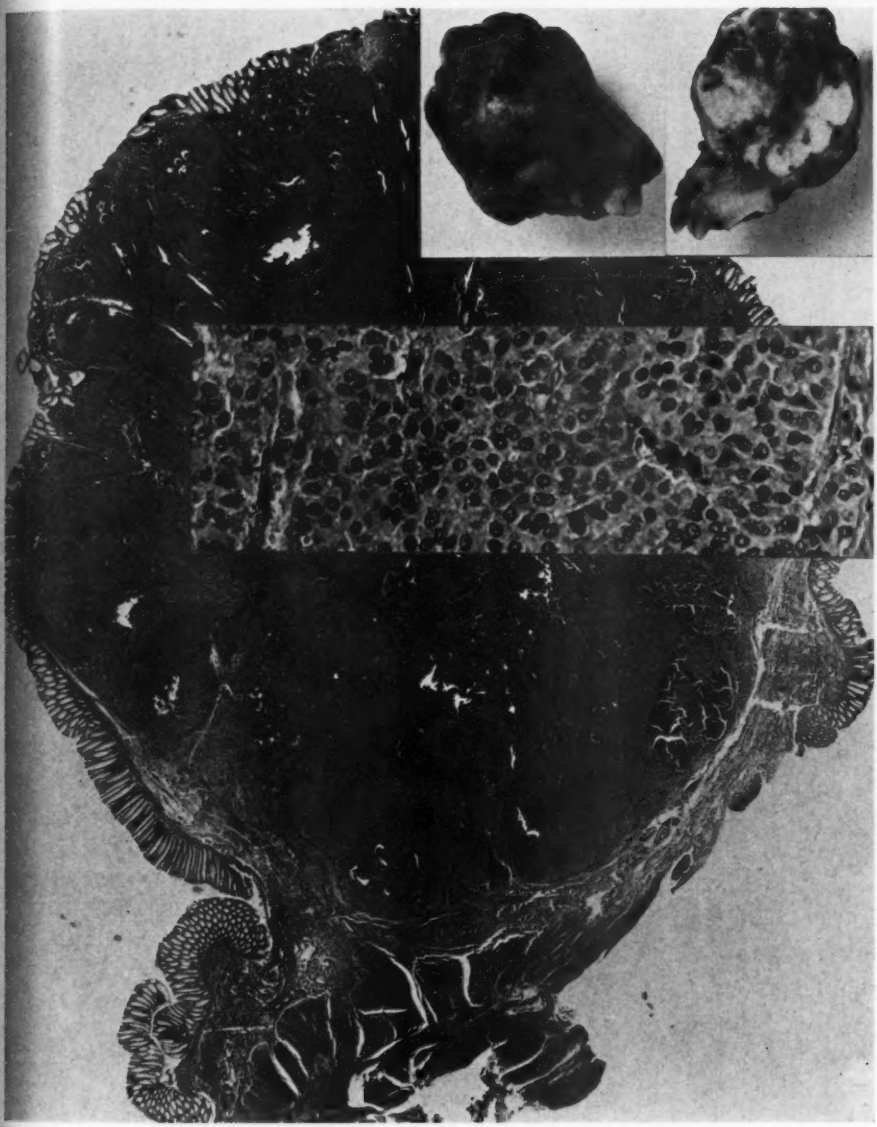
DESCRIPTION OF PLATES

Plate 154

Fig. 1. Case 1. The gross pictures in the upper right portion of the field show the external appearance of the excised specimen with the rectal mucosa elevated by the tumor, and the mottled surface of the nodular growth on cross section. The low-power photomicrograph made from a complete section through the entire tumor shows its situation in the submucosa, its invasion, and in two places its complete erosion of the mucosa, and, below, its failure to invade the muscular coats. It also shows a division of the tumor cells into huge, solid masses separated by slender fibrous bands. An enlargement of some of the cells forming one of these masses is inset.

Stout



Stout 1 Carcinoid Tumors of the Rectum

PLATE 155

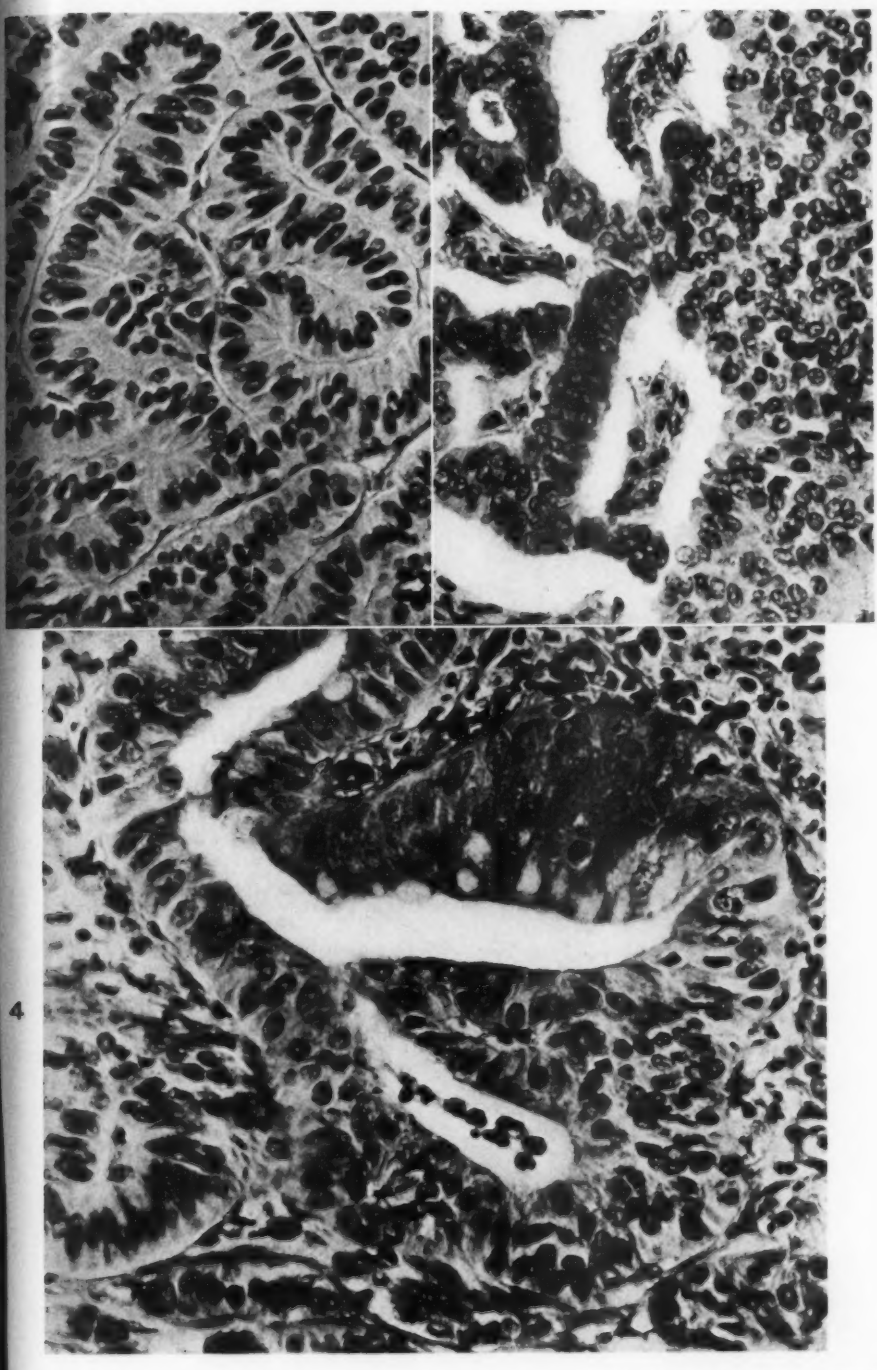
FIG. 2. A composite picture, slightly reduced from several photomicrographs. In the top row are three different basigranular cells in crypts of Lieberkühn from the rectum. They show the pyramidal or wedge shape of the cells, the elevated rounded nucleus differing from the cylindrical nuclei of the adjacent goblet cells, the granules in the basal pole and the lighter appearance of the cytoplasm surrounding the granules. In the middle row are characteristic polygonal cells from case 1 with groups of darkened granules eccentrically placed and only partly filling the cytoplasm. In the lower row at the left are columnar and prismatic tumor cells from case 1. One of these shows granules at one pole. This cord of cells was in the mucosa away from the large solid masses. At the lower right are columnar and polygonal cells from an appendiceal carcinoid tumor. Two of them show granules in one pole. All sections were stained with Masson's aniline blue-ponceau-acid fuchsin stain. In every instance the dark granules were stained a deep red in contrast to the other neutrally tinted cytoplasmic granules. × 1350.

Stout 2 Carcinoid Tumors of the Rectum

PLATE 156

FIG. 3. Case 2 (left); case 3 (right). The cells of case 2 are columnar and prismatic, arranged in ribbonlike sheets which twist and turn in every direction. The sheets are one cell wide and are separated by delicate reticulin fibers, sometimes bearing capillaries. In case 3 the ribbons have shrunk away from the fibrous framework, sometimes producing the appearance of tube formation. A point of junction is shown between ribbons of columnar cells and a mass of polygonal cells. Hematoxylin and eosin stain. × 416.

FIG. 4. Case 2. A distorted crypt of Lieberkühn lined by columnar goblet cells above toward the lumen of the colon, and by columnar tumor cells below in the direction of the submucosa. Masson's trichrome stain. × 416.



4

Stout Carcinoid Tumors of the Rectum

THE RENAL TUBULE (NEPHRON) AS AFFECTED BY MERCURY*

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Pictures and descriptions of renal tubular lesions have not been adequately specific with regard to the part of the tubule affected. Lack of specificity is understandable in view of the difficulties involved in ascertaining the precise locus and extent of such lesions. Indeed, this study would not have been undertaken without the compelling stimulus of convincing evidence of segmental structure and function of the tubule as revealed in various experiments and observations. Since among metallic poisons, mercury was accredited by Suzuki [1] with inducing a lesion in the terminal third of the proximal convolution, it seemed important to determine in requisite detail the fixed or variable site or sites of such tubular lesions as could be definitely attributed to the action of mercury.

This study was completed several years ago but owing to unavoidable circumstances the results obtained have thus far been published only in abstracts.[2,3] It would appear that segmental injury to the tubule is additional evidence of its structural and functional differentiation—especially functional—since the lesion caused by the action of mercury is restricted almost exclusively to portions of the proximal convolution which, in most vertebrates whose kidneys have been studied, have not been shown to be composed of histologically distinct segments. An aspect of such damage suggested the approximately simultaneous injection with the mercury of certain solutions in order to discover whether the site or degree of injury was changed thereby.

The data presented refer primarily to tubular damage induced by the action of mercuric chloride when it is injected into certain mammals and the frog. In another paper, functional changes relative to this damage will be discussed.

Material and Methods

The kidneys upon which the effects of mercury were studied were those of man, the rabbit, guinea pig and frog. Pieces of each kidney were fixed in Bouin's fluid and prepared for histological study while other portions of the same kidney were macerated in dilute HCl. Macerated pieces were dissected with glass needles under a microscope and each proximal convolution, as well as other portions of the tubule (in fortunate instances, the entire tubule) was towed to an area of the dish used in dissection where it was uncoiled as far as possible and

* Received for publication, January 22, 1942.

attached to a coating previously applied to the bottom of the dish. Damaged segments of tubules so isolated are detectable and measurable owing to a collapse of their walls or by their marked fragility. Histological study of similar tubules in the sectioned pieces supplemented the study of those which were isolated. Thus, by a combination of microdissection and histological study, the extent and degree of injury were determined.

The solutions used, expressed in grams per 100 cc., were as follows: mercuric chloride, 0.04; sodium ferrocyanide, 1; sodium thiosulfate, 1; ferric ammonium citrate, 1; urea, 20. The mammals were injected intraperitoneally and the frogs in the dorsal lymph sac.

Results of Experiments

Table I

Varying Degree of Necrosis in Segments of Proximal Convolutions, Tabulated by Eighths

Rabbits

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, killed, hours
1	4.4	..	..	..	..	..	T	T	..	384 d
2	3.3	..	..	..	..	..	T	T	..	3192 d
4	3.0	..	..	..	..	..	T	T	..	3216 k
5	4.1	..	..	..	T	T	T	T	..	88 d
6	3.4	..	..	..	..	..	T	T	..	360 d
9	4.1	T	T	T	T	T	T	T	..	162 d

T = total.

Rabbit no. 1 was given on the 1st, 3rd, 5th and 11th days, 2, 4, 5 and 5 mg. of mercuric chloride simultaneously with 5, 10, 12 and 12 cc. of Ringer's solution; likewise, nos. 2 and 4, except that sodium ferrocyanide and sodium thiosulfate, respectively, were injected instead of Ringer's solution. No. 5 was given one injection of 10 mg. of mercuric chloride with 25 cc. of sodium ferrocyanide and no. 6, on the 1st and 14th days, was given 10 mg. of mercuric chloride simultaneously with 25 cc. of sodium thiosulfate. No. 9 was given 10 mg. of mercuric chloride only.

Guinea Pigs

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, killed, hours
1	0.57	S	S	S	S	S	S	S	S	171 d
2	0.58	S	S	S	S	S	S	S	S	175 d
3	0.57	S	S	S	S	S	T	T	T	51 d
3A	0.61	S	S	S	S	S	T	T	T	285 d
4	0.51	..	..	..	..	..	..	..	..	452 d
5	0.46	T	T	T	T	T	T	T	T	30 d
6	0.52	P	P	P	T	T	T	T	T	40 d
7	0.37	..	..	P	P	T	T	T	T	304 d

P = partial; S = slight; T = total.

Guinea pigs nos. 1, 2 and 3, on the 1st and 3rd days, were given 1.6 and 1.2 mg. of mercuric chloride, respectively, with 4 cc. of sodium ferrocyanide simultaneously

and daily thereafter for 5 days (no. 1 only) and similarly 4 cc. of sodium thiosulfate with each injection of mercuric chloride (no. 3 only). No. 2 was given only the two injections of mercuric chloride. No. 3A, on the 1st, 3rd, 5th, 14th and 15th days, was given 2, 2, 3, 3 and 3 mg. of mercuric chloride with 3 cc. of Ringer's solution simultaneously on each of these 5 days. No. 4, on the 1st and 14th days, was given 1.6 and 1.2 mg. of mercuric chloride, respectively, with 4 cc. of Ringer's solution on these days and on the 3rd and 5th days also. Nos. 5, 6 and 7 were given one injection of 1.6 mg. of mercuric chloride with 4 cc. of sodium ferrocyanide (no. 5), with 4 cc. of sodium thiosulfate (no. 6) and 4 cc. of sodium ferrocyanide daily for 3 days (no. 7).

Human

No.	Weight in Kg.	1	2	3	4	5	6	7	8	Died, hours
4911	59	T	T	T	T	T	T	T	..	36
392292	61	P	P	P	P	T	T	T	..	360

P = partial; T = total.

No. 392292 was given several injections of sodium thiosulfate and 50 per cent glucose solution.

Rats

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
45	225	..	..	..	..	T	T	..	..	48 k
50	200	..	..	..	T	T	T	..	..	118 d
70	250	..	..	..	..	T	T	..	..	115 d
75	200	..	..	..	..	T	T	..	..	105 d
78	228	..	T	T	T	T	T	T	..	124 d
83	294	..	..	T	T	T	T	T	..	78 d
93	87	..	..	..	..	S	S	..	..	22 d
94	265	..	T	T	T	T	T	..	..	48 k
95	258	..	..	T	T	T	T	..	..	48 k
96	271	..	..	T	T	T	T	..	..	48 k

S = slight; T = total.

Rats nos. 45 to 96 were given 0.8 mg. of mercuric chloride only.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
92	214	..	..	T	T	T	T	..	..	72 k
97	332	P	P	P	P	T	T	..	..	72 k
98	400	..	..	P	P	T	T	..	..	72 k
99	330	..	..	P	P	T	T	..	..	72 k

P = partial; T = total.

Rats nos. 92 to 99 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of urea simultaneously and 3 cc. daily thereafter for 3 days.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
64	200	P	P	T	T	T	T	..	..	77 d
86	307	..	..	T	T	T	T	..	..	71 d
102	316	T	T	T	T	T	T	..	..	49 d
103	368	P	P	T	T	T	T	..	..	52 d
105	342	P	P	T	T	T	T	..	..	52 d
87	224	..	..	..	..	T	T	..	..	107 d
104	236	P	P	T	T	T	T	..	..	52 d
114	350	P	P	T	T	T	T	..	..	69 d
115	370	P	P	P	P	T	T	..	..	71 k
116	305	P	P	T	T	T	T	S	..	76 d
117	330	P	P	T	T	T	T	..	..	68 k
119	200	P	P	T	T	T	T	..	..	57 d

P = partial; S = slight; T = total.

Rats nos. 64 to 105 were given one injection of 0.8 mg., and rats nos. 87 to 119 0.6 mg. of mercuric chloride, with 4 cc. of sodium ferrocyanide simultaneously and 4 cc. daily thereafter for 3 days or until the rat died.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
107	307	P	T	T	T	T	T	P	..	54 d
108	339	P	T	T	T	T	T	P	..	58 d
109	325	P	T	T	T	T	T	..	..	49 d
106	334	..	P	T	T	T	T	..	..	62 k
110	359	S	S	P	P	P	P	P	..	56 k
112	348	P	P	T	T	T	T	S	S	60 d

P = partial; S = slight; T = total.

Rats nos. 107 to 112 were given one injection of 0.6 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of sodium ferrocyanide and ferric ammonium citrate (nos. 107 to 109); a second injection of 4 cc. of the mixture 24 hours later (nos. 106 and 110) and a third, like injection, 48 hours later (no. 112).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
30	256	..	..	..	..	T	T	..	..	75 d
100	370	S	S	T	T	T	T	..	..	56 d
101	320	S	S	P	P	P	T	..	..	75 k

P = partial; S = slight; T = total.

Rats nos. 30 to 101 were given one injection of 0.8 mg. of mercuric chloride with 2 cc. of ferric ammonium citrate simultaneously and 2 cc. daily thereafter for 2 days.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
68	260	P	T	T	T	T	T	..	..	114 d
69	155	P	T	T	T	T	T	..	..	83 d
77	250	P	T	T	T	T	T	..	..	104 d
84	230	P	T	T	T	T	T	T	..	70 d
85	226	P	T	T	T	T	T	..	..	77 d

P = partial; T = total.

Rats nos. 68 to 85 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of sodium thiosulfate simultaneously; 3 cc. 24 hours later (nos. 77 to 85) and a like amount 48 hours later (nos. 84 and 85).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
79	229	T	T	T	T	T	..	..	..	70 d
81	292	T	T	T	T	T	..	..	..	54 d

T = total.

Rats nos. 79 and 81 were given one injection of 0.8 mg. of mercuric chloride with 3 cc. of a mixture of equal parts of sodium ferrocyanide and sodium thiosulfate simultaneously and daily thereafter for 2 days.

Frogs

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
61	35	..	..	..	..	..	..	P	P	350 d
62	30	P	T	T	T	T	T	T	..	118 d
67	31	T	T	T	T	T	T	T	T	396 d
69	45	..	T	T	T	T	T	T	T	105 d
75	30	..	T	T	T	T	T	T	T	48 k
77	35	T	T	T	T	T	T	T	T	48 k
78	37	T	T	T	T	T	T	..	..	41 d

P = partial; T = total.

Frogs nos. 61 to 78 were given one injection of 0.6 mg. of mercuric chloride. No. 67 was given a like amount on the 7th day and no. 61 on the 7th and 10th days after the 1st injection.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
79	26	T	T	T	T	T	T	T	T	62 k
80	25	T	T	T	T	T	T	T	P	60 d

P = partial; T = total.

Frogs nos. 79 and 80 were given one injection of 0.4 mg. of mercuric chloride with 2 cc. of Ringer's solution simultaneously and 2 cc. of it daily thereafter for 2 days.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
81	25	P	P	P	P	S	S	S	S	28 k
82	25	T	T	T	T	T	T	T	T	38 k
83	30	P	P	T	T	T	T	P	..	51 k
84	25	T	T	T	T	T	T	T	T	51 k

P = partial; S = slight; T = total.

Frogs nos. 81 to 84 were given one injection of 0.4 mg. of mercuric chloride with 2 cc. of urea simultaneously and (nos. 83 and 84) 1 cc. of urea 24 hours later.

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
36	55	..	..	T	T	T	T	T	T	106 k
43	50	P	T	T	T	T	T	T	T	98 d
51	50	P	P	T	T	T	T	T	T	156 d
65	55	S	S	S	S	S	S	T	T	200 d

P = partial; S = slight; T = total.

Frogs nos. 36 to 65, in order, were given milligrams of mercuric chloride as follows: 0.56, 1.0, 0.6 and (no. 65 only) 0.6 on the 7th day after a like, 1st injection. Each frog was given, with the mercuric chloride, 3 cc. of sodium ferrocyanide simultaneously and 3 cc. of it daily thereafter for 4 days (nos. 36 and 43) and for 5 and 7 days, respectively (nos. 51 and 65).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
66	54	..	..	..	..	T	T	T	T	100 d
74	25	T	T	T	T	T	T	T	S	100 d
63	50	..	..	..	..	P	P	S	..	320 d
64	55	S	S	P	P	T	T	S	..	103 d

P = partial; S = slight; T = total.

Frog no. 66 was given 0.6 mg. of mercuric chloride with 4 cc. of sodium thiosulfate simultaneously and 4 cc. of it daily thereafter for 4 days. No. 74 was given 0.4 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of thiosulfate and urea simultaneously and 4 cc. of it daily thereafter for 3 days. No. 63 was given on the 1st, 6th and 12th days, 0.6 mg. of mercuric chloride and no. 64, one injection of 0.6 mg. of mercuric chloride with 4 cc. of a mixture of equal parts of sodium ferrocyanide and sodium thiosulfate, simultaneously, and 4 cc. of it daily thereafter for 10 days (no. 63) and for 5 days (no. 64).

No.	Weight in gm.	1	2	3	4	5	6	7	8	Died, killed, hours
15	50	..	..	S	S	T	T	T	T	49 k
40	48	T	T	T	T	..	..	..	..	107 k
58	45	..	..	..	..	..	..	P	P	153 k

P = partial; S = slight; T = total.

Frogs nos. 15 to 58, in order, were given milligrams of mercuric chloride as follows: 0.4, 0.56 and 0.4 with 3 cc. of ferric ammonium citrate simultaneously and with 3 cc. of it on the 2nd day (no. 15) and 3 cc. of it daily thereafter for 4 days (no. 40) and 6 days (no. 58).

Notes

These concern the proximal convolution since other portions of the tubule were not significantly damaged.

1. Rabbits and Guinea Pigs. The chief site of acute injury was a quarter between the fifth and terminal eighths (Figs. 1 and 4B—rabbits; Fig. 5—guinea pigs). Segmental damage in the kidney of the guinea pig was variable, as is shown in the tabulated data.

A unique finding and one of possible significance in connection with

the use of mercurial diuretics was the following: in two rabbits (nos. 2 and 4), in addition to necrosis of the cells of the portion indicated above, which 3 months after the initial injection of $HgCl_2$ appeared as though recently necrotized, the glomeruli were markedly damaged, their capsular spaces were enlarged and the neck of the tubule was occlusively constricted (Fig 4A). Such occlusion was not observed in the kidneys of the other rabbits studied or in those of the guinea pig and rat. It is possible that a like occlusion would have occurred in the kidneys of most of these animals if they had lived long enough after the $HgCl_2$ was injected.

2. Rats and Frogs. Acute segmental damage involved most frequently the second and third quarters while adjacent segments were less frequently involved (Figs. 2A and 6; 2B, 7 and 8—rats; 9, 10 and 11—frogs). In the frog, segmental damage varied considerably but the initial and terminal portions were less frequently damaged, the neck segment being the most resistant part of the tubule.

Remarks

1. Of unknown significance is the fact as shown here tabularly that only in the kidney of the guinea pig and frog does necrosis of the terminal portion of the proximal convolution occur frequently (guinea pig, 63 per cent; frog, 54.5 per cent) while necrosis of this portion in the kidney of the human, rabbit and rat has not been observed. Necrosis of the entire proximal convolution is rare despite the amount of $HgCl_2$ given or the period of the animal's survival thereafter. Of the animals studied, it has been observed in the kidney of only one guinea pig and in that of four frogs.

Frequency of Total Necrosis of Segments of Proximal Convolution, Tabulated by Eighths

		1	2	3	4	5	6	7	8
Rabbits	(6)	1	1	1	2	2	6	6	0
Guinea pigs	(8)	1	1	1	1	3	5	5	5
Rats	(43)	3	13	29	30	39	40	8	0
Frogs	(24)	9	13	16	16	18	18	15	13
Total	(81)	14	28	47	49	62	62	34	18

Figures in parentheses indicate the number of animals used.

The Degree of Segmental Damage of the Proximal Convolution, Tabulated by Eighths for All the Animals Studied

	1	2	3	4	5	6	7	8
None	33	28	18	16	9	7	36	56
Slight	10	10	6	6	7	5	7	6
Partial	25	16	11	11	3	2	6	3
Total	15	29	48	50	64	69	34	18

2. It has been noted by Crabtree [4] in the kidney of the mouse, and by others cited by her, that in several species of mammals the cells of the parietal layer of some glomerular capsules are normally cuboidal or columnar and that the number of such capsules increases in response

to certain factors.[5-8] In connection with the renal action of mercury a marked increase was noted in the number of glomerular capsules whose parietal layer was composed of cuboidal cells for varying distances from their junction with the proximal convolution to the vascular pole of the glomerulus (Figs. 3 and 7). These cells constituted in sections of renal corpuscles, structures resembling some of those designated as "epithelial crescents." The capacity for hypertrophy and hyperplasia seemed especially inherent in the cells of the parietal rather than in those of the visceral layer of the capsule. Marked damage of the cells of the initial portion of the proximal convolution was not accompanied by hypertrophy of those of the capsule's parietal layer. Hypertrophy or hyperplasia of the cells of the latter in the kidney of mercurialized animals may be correlated rather with segmental tubular damage than with obvious glomerular damage. A similar hypertrophy in animals treated with sex hormones may be contingent upon metabolic factors.

3. Mitotic figures have not been observed in the hyperplastic, parietal layer of the glomerular capsule nor has epithelial regeneration been observed in connection with the tubular lesions induced by $HgCl_2$. However, pertinent studies with regard to such regeneration and associated phenomena in uranium nephritis and in Bright's disease are those of Oliver and his co-workers.[9-12]

Generalizations

With regard to the animals studied and to the renal tubular damage resulting from the action of mercury, the data of this paper permit the following generalizations:

1. The initial and terminal portions of proximal convolutions are least and less frequently injured than are the intervening portions of such convolutions. The terminal segment (that segment terminating abruptly at its junction with the squamous-celled thin segment of the loop of Henle in mammals or with the cuboidal-celled, intermediate segment in frogs) is less frequently injured than is the initial segment except in the kidneys of the guinea pig and frog.

2. Segmental susceptibility of the proximal convolution to injury is least to greatest with abrupt transitions, from the end of the first eighth to the beginning of the terminal fourth or less. Necrosis of the cells of the most highly susceptible third quarter is often not accompanied by obvious injury to the remainder of this convolution. Also, in the cells of this quarter in the mammalian kidney, iron is normally demonstrable whether or not it has been previously injected into the animal.

3. The extent or degree of the necrotizing action of mercury appears

to be poorly correlated (a) with the amount of $HgCl_2$ injected; (b) with the size of the animal; or (c) whether the $HgCl_2$ is injected alone or with certain solutions.

4. The frog may be given ten to twenty times more $HgCl_2$ per body weight than may, with similar results, be given the mammal. The frog readily becomes edematous after the injection of effective amounts of $HgCl_2$. This does not occur in the mammal regardless of the amount of $HgCl_2$ injected.

5. The length of the lesion as measured in the third quarter of the proximal convolution varies with the length of the convolution in a given kidney.

Summary

1. Fifty-nine mammals and 24 frogs were injected with mercuric chloride. Some of these were injected with mercury alone or with mercury and certain other solutions in an effort to determine whether the site, extent, or degree of renal injury could thus be modified. The results of such injections are, in general, equivocal and those results indicative of some consequent and specific modification have not seemed to justify the large amount of tedious work that would be necessary in order to establish whether or not modification occurred.

2. The proximal convolution is the only subdivision of the renal tubule in which significant damage is observable after the injection into the various animals studied of usually lethal amounts of $HgCl_2$. Within this convolution a major aspect of such damage is the acute or total segmental necrosis of its cells. This necrosis is indicated below as percentages of involved eighths of the proximal convolution in the kidney of mammals and frogs.

	Eighths							
	1	2	3	4	5	6	7	8
Mammals	10	27	55	58	78	90	36	8.5 per cent
Frogs	38	55	67	67	75	75	63	55 per cent
	10	30	61	65	85	94.5	32	0 per cent

The figures of the last line give the average percentage for the human, rabbit and rat's kidney since, in contrast, the terminal portion of the proximal convolution is totally necrotic in the kidney of 65 per cent of the guinea pigs and in that of 55 per cent of the frogs.

3. Subsequent to the injection of $HgCl_2$, hypertrophy and/or hyperplasia of one-half or more of the outer layer of the glomerular capsule is common in the kidneys of all the animals studied except the rabbit where it is slight and infrequent.

4. Obvious injury to the cells of a part or of all of the distal convolution is rarely seen and there is functional evidence that such aspects of injury as may be observed are more apparent than real. The cells of the thin segment of the loop of Henle are too squamous to permit statements concerning their possible injury and those of the ascending limb are not appreciably damaged.

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DESCRIPTION OF PLATES

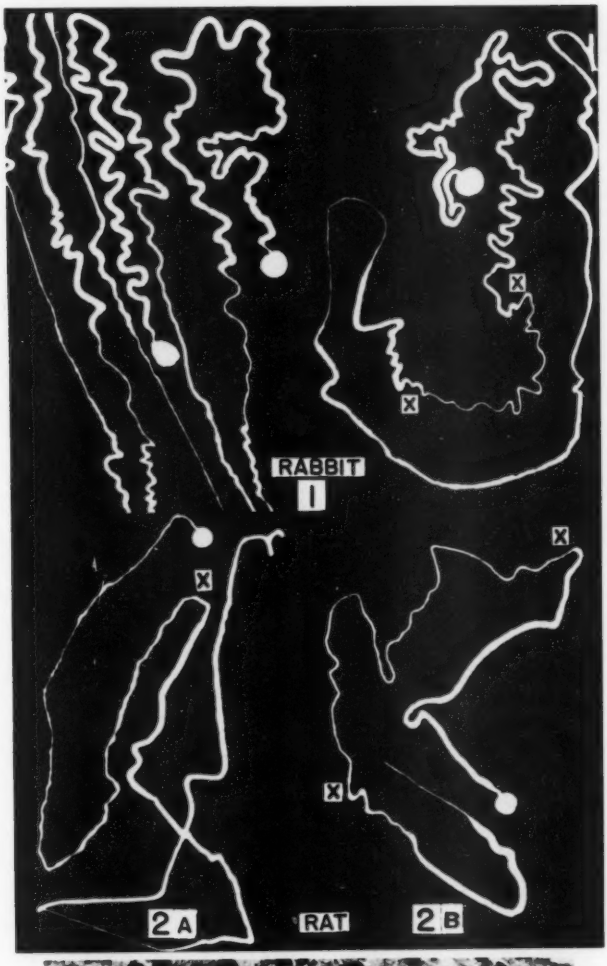
PLATE 157

(For all animals referred to by number in the figures, see tabulated data of the text.)

FIG. 1. Photomicrograph showing, left to right, the terminal portions of three and all of two proximal convolutions and one complete renal unit (glomerulus and tubule). These were isolated from the kidney of a rabbit which 4 months previously had been given, by injection, 16 mg. of $HgCl_2$ (rabbit no. 4). The site and extent of injury to the tubule at the right (see, also, Fig. 4B) are shown from X to X, and to the convolutions at the left, by their thin portions. The terminal filament of the third segment from the left is the normal, thin segment of the loop of Henle. × 50.

FIGS. 2A and 2B. In Figure 2A is shown a complete renal unit, and in 2B, a proximal convolution, which were isolated from the kidneys of rats nos. 79 and 95, respectively. The site and extent of total necrosis are shown in 2A, from the glomerulus to X, and in 2B, from X to X. × 50.

FIG. 3. Section of a human renal corpuscle in the kidney of a person dying of mercuric chloride poisoning. At the right is shown a crescentic structure resulting from hyperplasia of the cells of a portion of the outer layer of the glomerular capsule. At the left is shown an oblique section of the initial portion of a normal distal convolution. × 500.


RABBIT
1
2A
RAT
2B

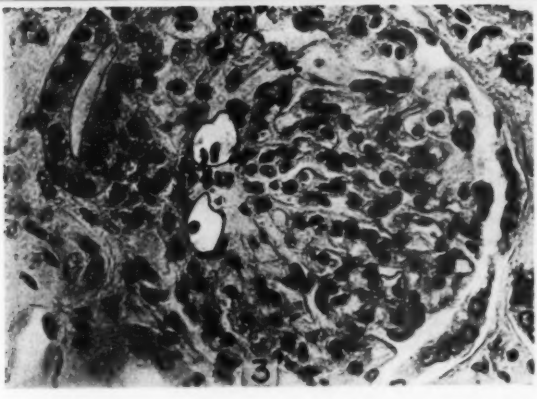
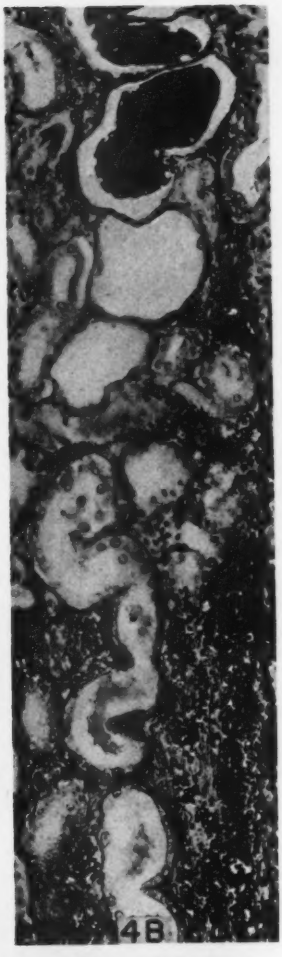

3

PLATE 158

FIGS. 4A and 4B. Sections of the cortex of the same rabbit's kidney as that from which Figure 1 was obtained. In A, the upper portions of proximal convolutions and two renal corpuscles are to be noted. The cells of these portions are not obviously damaged but the upper glomerulus is shrunken (the predominant condition of the glomeruli in this kidney and in that of rabbit no. 2). The outlet to the tubule from both glomeruli is constrictively occluded by connective tissue. In B, is shown the character of the lesion involving a part of the third quarter of the proximal convolution. × 300.

FIG. 5. A photomicrograph of composite sections of a guinea pig's kidney (guinea pig no. 7). The sections were so arranged as to show in a small area such features as otherwise would require several photomicrographs. In the center is a glomerulus and at its left, and continuous with its capsule, is the initial and here somewhat U-shaped segment of the undamaged first quarter of the proximal convolution. Below the glomerulus and at the right of the numeral is shown a looped portion of the partly necrotic second quarter. In the upper right bend of the latter is seen the rather abrupt transition between it and the totally necrotic terminal half. Portions of this and of another terminal half, similarly necrotic, extend across the top of the figure. × 200.

4A


4B

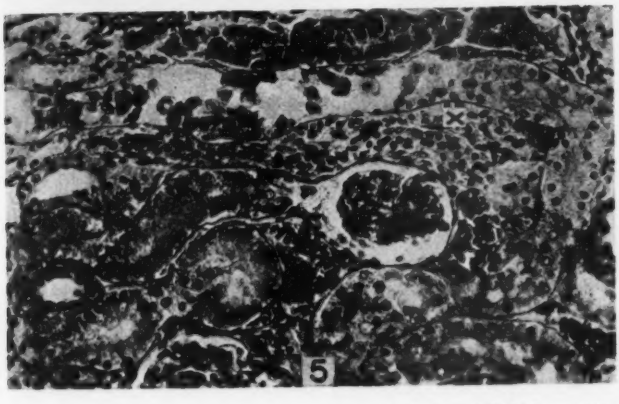

5

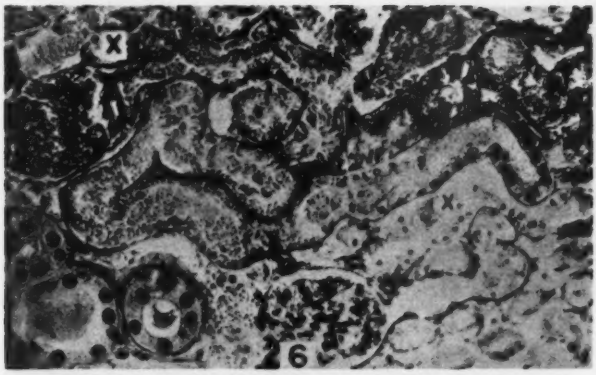
PLATE 159

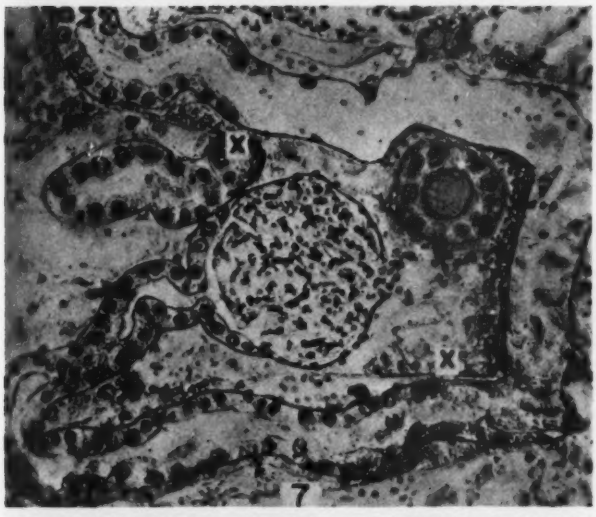
The figures of this plate are of sections of the kidneys of mercurialized rats. They were produced by combining several photomicrographs of different parts of the same proximal convolution within a given kidney so as to represent in miniature the chief site and extent of injury to this convolution in general throughout the kidney.

FIG. 6. Rat no. 79. Here is shown from X to X and also at the right of the lower glomerulus, the necrotic portion which is typical of the first five-eighths of the convolution and above the X at the right, the transition from the necrotic to the relatively nonnecrotic portion. × 150. At the lower left are shown three transections, the central one of which is of a distal convolution and the other two are of its ascending limb. × 300.

FIG. 7. Rat no. 95. This shows (a) at the left of the glomerulus the hypertrophic cells of the outer layer of the glomerular capsule; (b) the totally necrotic segment which, from X to X and abruptly continuous at both of its ends with partly necrotic segments, is typical of the second and third quarters; (c) a transection of a distal convolution at the right of the glomerulus. × 300.

FIG. 8. Rat no. 64 or 104. At the left of the glomerulus and beginning with its capsule, there are portions of a proximal convolution as follows: (a) partly necrotic and typical of the first quarter; (b) totally necrotic as shown at the right from the X above to the X below the glomerulus and typical of the second and third quarters; (c) the relatively undamaged terminal portion which, below the glomerulus, composes the upper left half of the loop and is continuous at its crest with that part of the thin segment which composes the lower left half. The latter is succeeded at the right by the initial part of the ascending limb. A transection of this limb is shown at the left of Y in the upper right portion of the figure. × 200.


X
X
6


X
X
7

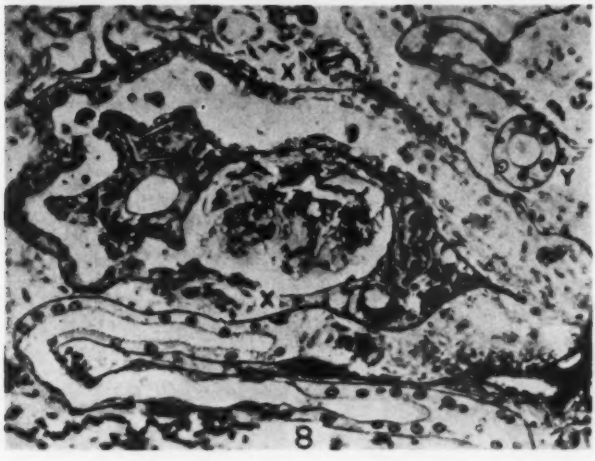

X
Y
X
8

Plate 160

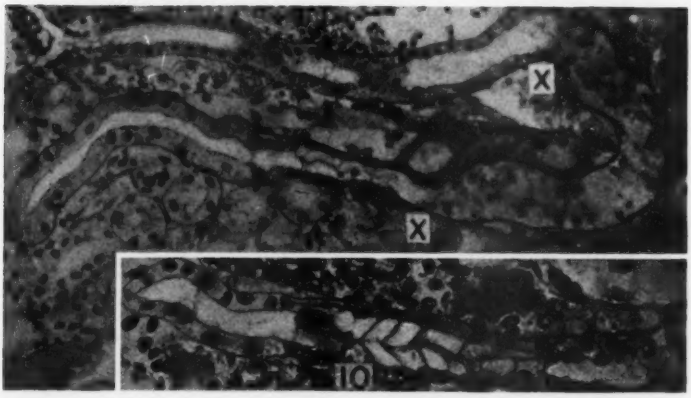
The figures of this plate were made as in Plate 159 by combining parts of photomicrographs of several sections of particular kidneys from mercurialized frogs so as to represent in each figure the typical aspects of tubules segmentally damaged by the action of mercury.

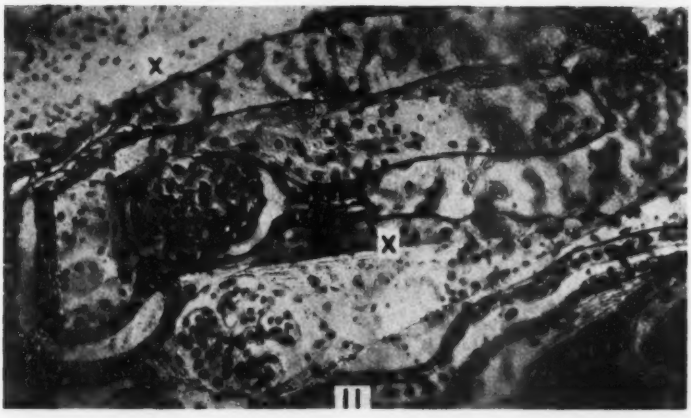
Fig. 9. Frog no. 64. Variable damage of the proximal convolution is indicated by typical segments of approximately equal lengths extending from the glomerulus at the upper right to the termination of the convolution beneath the upper X. The cells of the initial and distal portions are least injured as shown, respectively, from the glomerulus to the tubular constriction at the upper left and by the portion between and immediately to the left of X and X. Each of these segments constitutes about one-sixteenth of the entire convolution. From the constriction mentioned to the first X below, a degree of damage is shown which is typical of the first and second quarters except the initial sixteenth of the first quarter. The greatest injury, involving the cells of the third and most of the fourth quarter, is shown, respectively, by the upper and lower halves of the segment between X and X. Parts of two intermediate segments, *i.e.*, segments between the ends of proximal and the beginning of distal convolutions, are shown: one is beneath the upper X and the other extends from the numeral upward to the first slight curve where it becomes abruptly continuous with the initial portion of a distal convolution. The latter, and like convolutions throughout this and the kidney of other mercurialized frogs, are not significantly damaged despite the inadequacy of the figure as proof. × 300 (lower left quarter, × 200).

Fig. 10. Frog no. 69 or 75. There is shown from the upper left to the X at the upper right, the undamaged portion which is typical of the first eighth of the proximal convolution, and between X and X, the necrotic portion which is typical of the remainder of this convolution. Also, above the lower X and above and to the right of the numeral, there are parts of intermediate segments which are continuous at the left with initial portions of normal distal convolutions. These convolutions are, in general, normal in kidneys which have been damaged by mercury. × 200 and (inset) 350.

Fig. 11. Frog no. 82 or 84. From X to X in the upper right half, the total necrosis is typical of the entire proximal convolution except the normal neck segment. The latter is shown between the glomerulus and lower X. At the left of the upper X is an intermediate segment which is abruptly continuous at the extreme left with the initial portion of an undamaged distal convolution. In the lower right corner the sharply bent, terminal portion of a distal convolution joins a collecting duct. × 300.

X
X
9


X
X
10


X
X
11

THE ORIGIN OF COLLOID AND LIPOID DROPLETS IN THE EPITHELIAL CELLS OF THE RENAL TUBULES *

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The origin of colloid droplets in the lining cells of renal tubules has long been the subject of controversy. According to Aschoff,[1] colloid droplets are an expression of an inflammatory reaction of the cytoplasm (tubular nephritis). According to Fahr,[2] colloid droplets represent the secondary effect of a primary injury to the renal epithelium. Laas [3] distinguished between infiltrative and degenerative colloid droplets depending on whether they are due to storage of protein or disintegration of cytoplasmic protein. Terbrüggen [4] interpreted colloid droplets as an expression of disturbed secretion of tubular cells.

On the basis of results obtained in experiments with urodeles (*Salamandra maculosa*) Gérard and Cordier [5-8] and Lambert [9-11] concluded that colloid droplets represent protein material which has been taken up from the lumina of the tubules and stored by the tubular cells (athrocytosis). By analogy they interpreted the presence of colloid droplets in human renal disease as secondary to a functional change of the glomeruli permitting the passage of protein into the tubules.

Hein [12] and Kleier,[13] repeating and expanding the studies of Gérard and Cordier,[5-8] were able to confirm their findings. A comprehensive survey of the various theories and opinions concerning the formation, character and significance of colloid droplets in the renal tubules was given by Randerath.[14]

The problem was taken up by us in order to reinvestigate several points in the production of colloid droplets by employing a technic which made it possible to label the materials to be used. This seemed desirable because preliminary studies of the kidneys of five normal salamanders and three normal necturi had revealed unmistakable colloid droplets in the "open" and "closed" nephrons. These "natural" colloid droplets were quite distinct from the lipochrome granules and droplets occurring in the central portion of tubular cells of the "open" and "closed" nephrons.[6]

MATERIALS AND METHODS

We were unable to procure specimens of *Salamandra maculosa* and therefore employed *Salamandra punctata* (*Amblystoma punctatum*) and *Necturus maculatus* for our experiments. Both of these urodeles

* Received for publication, February 17, 1942.
† Deceased.

are equipped with nephrostomial canals connecting the peritoneal cavity with the "open" nephrons, so that the experimental conditions are comparable to those in *Salamandra maculosa*. The kidney of necturus is especially well suited for this work because of the single segmental "open" nephrons which are easily identified.

The various proteins to be used were coupled with the disodium salt of 2-naphtol-3:6 disulfonic acid (R salt)* according to the procedure described by Kabat and Heidelberger.[15] After having twice precipitated the dye-protein compounds with acetic acid, the precipitate was dissolved in saline of pH 7 and then dialyzed against saline of the same pH until no more uncombined dye passed through the membrane. One per cent by volume of a 1 per cent solution of merthiolate was added to each preparation as a preservative. Nitrogen determinations were made with the micro-Kjeldahl method in duplicate.

The protein-R salt compounds are quite stable and can easily be identified, grossly as well as microscopically, by their intense red color.

The preparations used in the experiments were as follows:

Neopeptone (Difco Laboratories, Detroit, Michigan)—R salt, 0.2 mg. N per cc.
Egg albumin (thrice recrystallized)—R salt, 6.8 mg. N per cc.
Serum albumin (dog)—R salt, 0.3 mg. N per cc.
Serum albumin (necturus)—R salt, 0.2 mg. N per cc.
Serum globulin (dog)—R salt, 0.3 mg. N per cc.
Serum globulin (necturus)—R salt, 0.4 mg. N per cc.
Serum globulin (cat)—R salt, 1.8 mg. N per cc.
Casein—R salt, 1.1 mg. N per cc.
Diazotized R salt alone, 0.12 per cent.

In addition to R salt, preparations of dyes of the malachite green series[16-18] were coupled with various proteins. In this manner protein-dye compounds of green color (m-malachite green) and of blue color (p-malachite green) were obtained. The dyes were coupled to the proteins according to the method of Heidelberger:[19] 0.34 gm. of m- or p-NH_2 leukomalachite green was dissolved in 25 cc. of H_2O to which were added 2 cc. of 1:1 concentrated HCl. After chilling, the dye was diazolized with 0.08 gm. of $NaNO_2$, dissolved in 5 cc. of H_2O. 17 cc. of a suspension of $PbCO_2$ (containing 14.6 mg. per cc.) were added with constant stirring. After a few minutes, 0.2 gm. of Na_2SO_4 were added, after which the solution was centrifugated to remove the lead sulfate.

290 cc. of a solution of NH_3 free thrice recrystallized egg albumin containing 10.4 mg. protein per cc. were diluted to 500 cc. and chilled. 25 cc. of 2N Na_2CO_3 were added and 70 cc. of the above-mentioned diazo solution were allowed to run in drop-wise with constant stirring. A similar technic was used in the preparation of other protein-dye compounds.

The following protein-dye compounds were prepared by this procedure:

Neopeptone (Difco Laboratories, Detroit, Michigan)—p-malachite green, 0.86 mg. N per cc.
Crystallized egg albumin—m-malachite green, 0.7 mg. N per cc.
Serum globulin (sheep)—m-malachite green, 0.82 mg. N per cc.
Casein—p-malachite green, 1.3 mg. N per cc.

* The R salt was supplied through the kindness of Dr. L. M. Crossley of the Calco Chemical Company.

For certain experiments human sera, unlabelled by coupling with dyes, from cases of lipoid nephrosis, containing 950 mg. per cent and 860 mg. per cent respectively of cholesterol and horse serum (total protein, 12.8 mg. N per cc.; albumin, 5.2 mg. N per cc.; globulin, 7.4 mg. N per cc.) were used.

Groups of animals were injected intraperitoneally 1 to 4 times with 0.5 cc. of the various preparations and the animals were then sacrificed after different periods of time ranging from 2 to 328 days. The tissues were fixed in Zenker's fluid containing 5 per cent of glacial acetic acid, and in a 4 per cent solution of formaldehyde. Paraffin sections of animals having received R salt preparations were stained with hematoxylin only, while those with multiple dye injections were examined unstained. Portions of formaldehyde-fixed kidneys were cleared according to the method of Spalteholz [20] so that the entire length of the tubules of the "open" nephrons could be studied.

a. The Distribution of Protein-R Salt Compounds in the Organs of Urodeles After Intraperitoneal Injection

All protein-R salt preparations mentioned above, including peptone, placed into the peritoneal cavity of 6 *Amblystoma punctatum* and 24 *Necturus maculatus*, produced intensely red granules or droplets in the lining cells of the tubules of the "open" nephrons only. In no instance were colored particles seen in the cells of the "closed" nephrons (Figs. 1 and 2). In addition, colored granules were usually present in the Kupffer cells and occasionally in some of the reticuloendothelial cells of the spleen. Sometimes phagocytic cells, holding colored granules, were seen in the peritoneal layer of abdominal organs. Necturi, injected with necturus serum albumin or globulin-R salt preparations, showed colloid droplets in the tubular cells of the "open" nephrons but did not show colored granules in the Kupffer cells. Preparations of segments of necturus kidneys cleared according to the method of Spalteholz [20] never revealed colored material in any of the "closed" nephrons while the "open" tubules were always clearly defined by their intense red color.

Similar results were obtained after injections of proteins coupled with m- or p-malachite green.

Colored granules and droplets were also seen in the "open" nephrons after the intraperitoneal injection of diazotized R salt alone. This result was quite puzzling until it was found that the fluid normally present within the peritoneal cavity of the necturus contained 0.65 per cent protein. The R salt injected had combined with this protein and then produced colored colloid droplets. The presence of protein-containing peritoneal fluid easily explained the occurrence of "natural" colloid droplets seen in untreated animals.

b. The Relationship Between the Character of Colloid Droplets in the Renal Epithelium and the Substances Employed to Produce Them

The size of colloid droplets varied according to the substances employed as well as to the time elapsing between the intraperitoneal injection and observation. Newly formed colloid droplets were always large, rather faintly tinted and occupied the supranuclear zone of the cytoplasm of the tubular cells. After some time they became smaller, darker in color and were situated in the middle and basal portions of the cytoplasm where they attained a certain relative uniformity. The constantly largest colloid droplets were seen in experiments with globulins (Fig. 3) and with a 20 per cent suspension of casein-R salt in saline, while solutions of casein-R salt produced rather small granules and droplets. The most uniform granules of small size were observed with egg albumin-R salt preparations. Serum albumin produced both large and small droplets as did peptone. There was no constant relationship between the size of the colloid droplets and the molecular weight of the substances employed, which varied from about 20,000 (peptone) to over 100,000 (globulin, casein). We were unable to confirm the statements of Lambert [11] and Hein [12] that intraperitoneal injection of split products of proteins causes formation of tiny droplets in the proximal portion of the convoluted tubules of the "open" and "closed" nephrons. In our experiments with peptone-R salt preparations no colored droplets were seen in the cells of the "closed" nephrons, although the size of the peptone molecule would permit its passage through the glomerular filter.

c. The Site of Storage of Colored Particles within the "Open" Nephrons in Relation to the Molecular Weight of the Substances Employed

According to Gérard and Cordier,[8] Lambert [11] and Hein [12] the site of storage of proteins or dyes within the nephrons depends on the molecular size or dispersion of the substances employed: the larger the molecule, the more distally it is stored.

The validity of this statement was tested in several ways:

(*1*) *The Site of Storage of Protein-R Salt Compounds of Different Molecular Weights.* Twenty-seven necturi were injected intraperitoneally 1 to 4 times with 0.5 cc. of different protein-R salt preparations varying in molecular weight from about 20,000 to over 100,000, and were then sacrificed after a varying length of time. Segments of kidneys of such treated animals were cleared according to the method of Spalteholz [20] so that the length of the tubules of "open" and "closed" nephrons could be seen. In all of these preparations the tubules of the

"open" nephrons stood out clearly, due to their red color (Fig. 4). The coloration, which was due to the presence of red granules or droplets within the lining cells, began at the point where the nephrostomial canal joins the lumen of the proximal convoluted tubule, and ended at the junction between the distal convoluted tubule and the collecting tubule (wolffian duct). Sometimes a faint coloration extended into the neck of the proximal portion of the convoluted tubule and rarely was color seen in the space of Bowman of the glomeruli of the "open" nephrons. In no instance was there any specific location of storage within the "open" nephrons. The intensity of the color varied according to the time elapsing since the last injection and seemed proportional to the amount of material taken up by the lining cells. There was, however, no difference in the intensity of color in different portions of the "open" nephrons. As stated above, the "closed" nephrons were always free of colored particles.

(2) *Multiple Protein-Dye Injections.* Four series of experiments were performed with groups of three necturi each. In two series, mixtures of three proteins coupled with different dyes were injected intraperitoneally, while in the other two series, three protein-dye compounds were injected separately, spacing the injections 1 week apart.

The combinations of materials used in these experiments were as follows:

Series 1. Peptone-p-malachite green, egg albumin-R salt, sheep serum globulin-m-malachite green. One cc. of a mixture of these three compounds was injected on 3 consecutive days.

Series 2. One cc. of each of the three compounds used in series 1 was injected separately with an interval of 1 week between injections.

Series 3. One cc. of a mixture of the following dye-protein compounds was injected on 3 consecutive days: egg albumin-m-malachite green, cat serum globulin-R salt, casein-p-malachite green.

Series 4. One cc. of each of the compounds used in series 3 was injected separately, spacing the injections 1 week apart.

In addition, a few necturi were injected with similar dye-protein combinations, spacing the individual injections approximately 1 month apart. The animals were sacrified after periods of time varying from 2 days to 36 days after the last injection.

Histologic preparations of the kidneys of necturi of series 1 and 3 showed uniformly colored granules and droplets in the cells of the tubules of the "open" nephrons. The color was the resultant of the mixture of the three colors—red, green and blue. Kupffer cells contained small granules of the same color. No colored material was seen in any of the tubules of the "closed" nephrons. Cleared segments of

the kidneys showed the entire length of the tubules of the "open" nephrons uniformly affected.

Histologic preparations of the kidneys of series 2 and 4 showed granules and droplets of different colors in the cells of the tubules of the "open" nephrons only. The droplets having the color of the protein-dye compound injected first were always seen in the basal portion of the cells, those of the last injection towards the lumen and the second one in the central portion of the cells. Individual tubules showed uniform distribution in all parts of the nephron but there were differences in the number of droplets of different color in different nephrons. From the outcome of these experiments, it was concluded that there is no preferred site of storage of protein particles of different character in different parts of the tubules of the "open" nephrons.

In order to prove that in a mixture of three protein-dye compounds the dyes were inseparably attached to their respective proteins, the mixtures used in series 1 and 3 were studied in the electrophoresis apparatus of Tiselius.[21] The mixtures were dialyzed against 0.02 mol. phosphate buffer containing 0.15 mol. NaCl, pH 7.51 and were then run for 2 hours in a standard cell under standard conditions. The electrophoretic mobilities of the boundary lines were studied and the pattern was photographically recorded by means of Longsworth curves[22] and by cylindrical lens curves[23,24] using special lamps and filters to record the different colors of the mixture.

Both preparations showed three boundaries in the ascending and descending limb. Series 1 showed a fast-moving blue component, a slow-moving green component, while the middle portion was of a composite purple color. Series 3 showed a faster moving greenish blue component, a very slow red component, while the middle portion was again of a composite purplish color. The mobilities of the boundaries corresponded to those of the substances contained in the mixtures. The results obtained in these experiments indicated that protein-dye compounds had retained their individual character within the mixture.

d. The Fate of Colloid Droplets

According to Kleier,[13] colloid droplets appear in the cells of the tubules of the "open" nephrons 2 days after the injection of protein material into the peritoneal cavity and disappear in summer animals 10 days, in winter animals 17 days, after the last injection. Concerning the mechanism of their disappearance, Hein[12] speculated whether metamorphosis (*Umbau*) or decomposition (*Abbau*) of the colloid droplets takes place, while Randerath[14] and Kleier[13] spoke of digestion and splitting. Kleier described a change in position of the colloid

droplets within the cells, first from the supranuclear zone to the infranuclear zone and then back towards the lumen just before their disappearance; he also pointed out a vacuolar change of the cytoplasm after the disappearance of colloid droplets. According to Lambert,[9] colloidal dyes taken up by renal epithelial cells disappear very slowly, if at all, and this is contrasted by Hein with the rapid disappearance of colloid droplets.

The reinvestigation of the fate of the colloid droplets was complicated by two factors: the occurrence of spontaneous colloid droplets, on the one hand, and on the other, the possible interference of the coupled dye with the digestion of colloid droplets. For this reason we studied the fate of colloid droplets produced by labelled as well as unlabelled materials, and compared the results.

Ten necturi were injected intraperitoneally with 0.5 cc. of horse serum on 4 consecutive days and the animals were sacrificed at intervals 7 to 67 days after the last injection. Small series of sections of the kidneys were stained with hematoxylin and eosin and were thoroughly examined for the presence of colloid droplets in the "open" nephrons.

Unmistakable colloid droplets of various sizes were present in the tubular cells of the "open" nephrons in all. Their number varied but this did not seem to be definitely related to the time elapsed. The largest number of colloid droplets in this series of experiments was seen in animals sacrificed 32 and 39 days after the last injection of serum. No signs of digestion of colloid droplets could be made out; there were, however, a few vacuoles within the cytoplasm of the tubular cells of the "open" nephrons. Frozen sections stained with scharlach R showed these to be fat droplets.

In the experiments conducted with the above-mentioned protein-R salt (sections a and b) colored colloid droplets were present in the tubular cells of the "open" nephrons in all instances from 2 days to 328 days after the last intraperitoneal injection. The character of the droplets changed during this time. In the earliest stage colored protein casts were present in the lumina of the tubules. Some of this amorphous material was then absorbed by tubular cells and segregated into large, faintly tinted colloid droplets just beyond the brush border of the cells in the supranuclear zone of the cytoplasm. Soon afterwards the droplets were moved towards the base of the cells where they became smaller and deeper red in color. Often the supranuclear zone was then free from colored colloid droplets but showed large, untinted, "natural" droplets (Fig. 5). In animals killed within 2 to 3 weeks after the last injection, some of the cells were tremendously distended by large

colloid droplets, sometimes causing rupture of the cell membrane (Fig. 6). Occasionally, colored droplets were seen in some of the nuclei of tubular cells. Desquamation of cells distended by colloid droplets was not infrequently observed, especially during the earlier stages, and such discharged cells, with colloid droplets still within their cytoplasm, could be seen within the lumina of tubules. Depending on the amount of coupled material still present in the peritoneal cavity, the regenerated cells showed only a few colored granules or untinted droplets, or none at all.

After about 1 month the colored granules and droplets were all concentrated in the basal portion of the cells and sometimes formed dense segregated lumps. At about the same time colored granules and droplets were observed in cells of the stroma surrounding the tubules of the "open" nephrons (Fig. 7). At first these cells were usually situated just beyond the basement membrane of the tubules and the number and the size of the individual granules, which they contained, varied from a few tiny particles to large colloid droplets or masses of deeply stained red material. The number of these phagocytic cells increased and they seemed more widely scattered through the intertubular stroma the longer the animals were allowed to live. Not infrequently such cells were then seen within lumina of peritubular capillaries and lymphatics, which suggested that they were capable of locomotion.

Since there seemed to be a probable relationship between the labelled colloid droplets within the cells of the tubules and the colored material in the cells outside the tubules, a thorough search was made to find any means of transportation of the tagged proteins through the basement membrane. In several instances close study of sections (stained with a modified Mallory stain to bring out the basement membrane of the tubules) strongly suggested that there exist tiny pores in the basement membranes through which granules can pass and be then taken up by phagocytic cells of the peritubular stroma (Fig. 8).

Although colored granules or droplets were still present in the tubular cells almost 1 year after the last peritoneal injection of labelled materials, their number decreased definitely in the course of time. This was attributed to desquamation of entire cells as well as to a discharge of granules through pores of the basement membranes. There was no evidence of splitting, nor was there ever seen a reverse movement of droplets from the base of the cells towards the lumen of the tubules. The colored granules in the Kupffer cells also remained visible throughout the duration of the experiments.

In order to study the effect of an enzyme on protein coupled with R salt *in vitro*, 10 per cent by volume of a 2 per cent solution of trypsin (Fairchild) was added to a casein-R salt solution of pH 8. An analysis

of this mixture revealed 1.91 mg. total nitrogen per cc.; 0.33 mg. of nonprotein nitrogen per cc.; 1.07 of noncoagulable nitrogen per cc., and 0.41 mg. amino nitrogen per cc. Twenty-seven cc. of the mixture were placed in a cellophane bag which was surrounded by 40 cc. of a solution of phosphate buffer of pH 8 and kept at 37° C. After 24 hours the total nitrogen content of the fluid within the bag had fallen from 51.4 mg. to 25.8 mg. while that of the outside buffer had risen to 25.6 mg. The nonprotein nitrogen of the digested material was then 2.16 mg. per cc., the noncoagulable nitrogen 2.7 mg. per cc. and the amino nitrogen had risen to 6.9 mg. per cc. A parallel experiment with untreated casein gave comparable results. Several such experiments gave similar results, which indicated that the coupling of the casein to R salt did not interfere with the digestion by trypsin *in vitro*.

The effect of trypsin and pepsin on colloid droplets produced by coupled and native protein was studied by subjecting unfixed frozen sections of kidneys to solutions of the enzymes on the warm stage at 37° C. There was fading out of the color from the colloid droplets after 2 hours following trypsin digestion, while the colloid droplets remained visible for 24 hours. Pepsin neither affected the colloid droplets nor their color.

The Effect of Intravenous Injection of Diazotized R Salt and Protein-R Salt Preparations

One cc. of diazotized R salt was injected into the inferior vena cava of two necturi under anesthesia and the animals were bled to death 6 hours and 16 hours, respectively, after the injection. The serum of the necturus sacrificed after 6 hours showed the deep red color of the R salt; that sacrificed after 16 hours was of light red color. There was no hemolysis in either serum. Sections of the organs of the animals revealed bright red granules in phagocytes in most of the glomeruli and rare, faintly colored granules in the Kupffer cells. No trace of the dye was found in any of the renal tubules of the "open" and "closed" nephrons. The color of the serum showed that the diazotized R salt had combined with the serum proteins but had not passed into the renal tubules.

One cc. of casein-R salt solution was injected under anesthesia into the inferior vena cava of a necturus and the animal was sacrificed 4½ hours afterwards; likewise, 0.3 cc. of the same solution was injected into an *Amblystoma punctatum* and the animal killed 56 hours afterwards. Tiny red granules and faintly colored droplets were found in tubular cells of "open" and "closed" nephrons in both. In another set of experiments two necturi were injected intravenously with 1 cc. of egg albumin-R salt solution and the animals were sacrificed 72 hours

and 1 week afterwards. Abundant colored granules were present in the Kupffer cells 72 hours after the injection and some of the glomeruli contained similar granules in phagocytic cells. The lining cells of the "open" and "closed" nephrons showed small red granules. No evidence of the dye-protein was seen in the kidney 1 week after the injection, while the Kupffer cells showed definite colored granules.

Because of the unexpected passage of casein-R salt through the glomerular filter in urodeles, a study was undertaken to follow the fate of various protein-dye preparations after intravenous injection in mice.

The following solutions were employed: peptone-R salt, egg albumin-R salt, serum albumin (dog)-R salt, serum globulin (cat)-R salt, casein-R salt. Series of five mice each were injected with 0.5 cc. intravenously and the animals sacrificed ½ hour, 1 hour, 2 hours, 3½ hours and 24 hours afterwards.

With the exception of mice having received peptone-R salt, the kidneys of all animals showed tiny, bright red granules in the cells of the proximal convoluted tubules which were more numerous the longer the animals were allowed to live. In the animals killed ½ hour after injection, there were but a few granules in the epithelial cells of the most proximal portion of the tubule at the point of junction with the space itself. The longer the mice were allowed to live after the injection, the farther down the granules extended into the more distal portions of the proximal convoluted tubules. They were most numerous after 24 hours. The Kupffer cells contained similar bright red granules in all experiments including peptone and the granules seemed more numerous in the animals that had lived longer. Although peptone-R salt was excreted into the urine, as evidenced by the dark red color, no colored granules were seen in renal cells. A second series of five mice injected similarly with peptone-R salt showed no granules in the renal epithelium, although the Kupffer cells contained colored material. Studies on the renal excretion and tubular reabsorption of dye-protein compounds in several mammalian species are now under way and will be reported elsewhere.

The Effect of Intraperitoneal Injection of Lipoids on the Renal Epithelium

The lining cells of the "open" and "closed" nephrons of necturus and *Amblystoma punctatum* contain normally a few basally located fat droplets usually situated in the loops of the distal convoluted tubules. Doubly refractive lipids were never encountered in normal animals and therefore preparations, rich in cholesterol, were used to label the materials employed in these experiments. After five intraperitoneal injections of 0.5 cc. each of serum from cases of lipoid nephrosis, the animals were sacrificed from 2 days to 300 days after the last injection.

Six salamanders and 15 necturi were studied in this manner. In addition to paraffin sections stained with hematoxylin and eosin, frozen sections were cut and stained with scharlach R; unstained frozen sections were examined under the polarizing microscope.

In paraffin sections stained with hematoxylin and eosin the cells of the "open" nephrons were large, pale-staining and vacuolated (Fig. 9). Frozen sections stained with scharlach R revealed large numbers of fat droplets of various sizes in the cytoplasm of the cells throughout the entire length of the "open" nephrons, which had the tendency to accumulate in the basal portion of the cells (Fig. 10). Abundant doubly refractive cholesterol crystals and Maltese crosses were demonstrated under the polarizing microscope (Fig. 11). Cells containing fat droplets but no doubly refractive particles were encountered in the stroma surrounding the tubules of the "open" nephrons; their number varied and corresponded generally to the length of time which had elapsed since the last injection. In addition to the fat droplets, the tubular cells of the "open" nephrons showed abundant large colloid droplets. The number of fat droplets in the cells of the "closed" nephrons was not increased and double refraction was never encountered.

The mechanism of the absorption of lipoids from the tubular lumina was similar to that observed after intraperitoneal injection of proteins and formation of colloid droplets. In early cases globular casts of different sizes made up of lipoid-containing serum were seen in the tubular lumina. These were then split up into small droplets beyond the brush border of the lining cells. At first the lipoid droplets filled the entire cytoplasm of the lining cells and later accumulated in the basal portion.

Abundant lipoid droplets, which partly were doubly refractive, as well as colloid droplets, were present in the cells of the "open" nephrons of a necturus 300 days after the last injection of nephrotic serum. Fat droplets, sometimes showing double refraction, were also encountered in the Kupffer cells of the injected animals and in some cases the liver cells seemed to contain more fat than normal.

Discussion

The results of the experiments on urodeles with dye-protein compounds definitely relate the colloid droplets in the "open" nephrons to the various materials injected intraperitoneally. The presence of "natural" colloid droplets was by no means a rare occurrence in our experience, so that the results of previous studies with unlabelled materials cannot be correctly evaluated. The presence of protein in the peritoneal fluid of normal animals adequately explained the occurrence of "nat-

ural" colloid droplets in the "open" nephrons, and it may be the normal function of these tubules to reabsorb valuable substances which otherwise would be lost. Colloid droplets were seen 2 days after the intraperitoneal deposition of tagged proteins and their number increased as long as there remained material to draw from. After 1 to 2 weeks the cells usually were filled to capacity so that rupture of the cell membrane sometimes occurred. Desquamation and sloughing of lining cells were also frequently encountered, indicating damage by overloading with colloid droplets. Colloid droplets were formed only within cells from amorphous protein casts within the tubular lumina. The material was absorbed by the lining cells and at first segregated into large globular masses beyond the brush border. Soon these were changed into well rounded colloid droplets which still occupied the supranuclear zone of the cytoplasm. Gradually the droplets became smaller and were moved towards the basal portion of the cells, where they remained. The decrease in size and corresponding increase in color suggested condensation and dehydration of the labelled materials within the cells. The question of the ultimate fate of colloid droplets has to be left open; on the one hand, studies with unlabelled proteins are unsatisfactory because of the occurrence of "natural" colloid droplets, while, on the other hand, the interference of coupling of dyes on splitting and digestion of proteins by cells cannot be ruled out with certainty. The absence of "natural" colloid droplets in the "open" nephrons of many of the animals suggests that colloid droplets can be split and digested, depending perhaps on the amount of protein within the cells. Actual evidence of digestion of colloid droplets within cells was not observed. The vacuoles which Kleier [13] described and which were also seen in our material, occurred only after injections of whole serum and were found to represent fat. Although there was evidence that protein particles and fat droplets could pass the basement membrane of the tubules through small openings, it is rather questionable whether this mode of passage plays an important rôle in the elimination of colloid droplets from the lining cells, since abundant colored colloid droplets were still present in most of the cells 1 year after the last injection. Phagocytosis and storage within cells of the "open" nephrons were not influenced by the kind of protein used. Protein substances of homologous and heterogenous species were taken up in the same manner and remained in the cells for the same length of time.

In no instance was the intraperitoneal injection of coupled protein material followed by the appearance of colored colloid droplets in the lining cells of the "closed" nephrons, even when preparations were used whose molecular size would ordinarily permit passage through the glomerular filter. Although the Kupffer cells showed colored granules in

practically all experiments, these cells were apparently able to retain the materials reaching the liver, perhaps by means of lymph channels, so that they were not disseminated throughout the general circulation. Intravenous injection of coupled egg albumin and casein, however, caused formation of colored particles in the cells of both the "open" and "closed" nephrons, indicating passage of these substances through the glomerular filter.

Laparatomy wounds of necturi and salamanders which were made to inject materials into the vena cava had no tendency to heal. Therefore, mice were used to follow the fate of labelled proteins injected intravenously. Surprisingly, all proteins employed, including dog serum globulin and casein, partly passed through the glomerular filter and were reabsorbed by the lining cells of the proximal convoluted tubules and stored in the form of fine granules and droplets with the exception of peptone, which passed into the bladder urine.

Preparations of serum globulin, injected intraperitoneally, had the tendency to produce rather large colloid droplets in the "open" nephrons. Otherwise there was no constant relationship between the size of the colloid droplets and the substances employed to produce them. In general, the size of the droplets depended more on the time which had elapsed since the peritoneal injection than on the substance employed.

No selective site of storage for any of the proteins within the "open" nephrons could be demonstrated. The entire length of the tubules was equally affected, no matter which preparation was used, and proteins of different molecular weight coupled with dyes of different color were stored in the same cells throughout the "open" nephrons.

The results of experiments conducted to study the phagocytosis and storage of lipids in the cells of the "open" nephrons confirmed those reported by Gérard and Cordier.[7] Fats and lipids never appeared in the "closed" nephrons and the fat droplets and cholesterol remained in the cells of the "open" nephrons as long as did the colloid droplets.

The colloid droplets and fatty changes produced experimentally in urodeles are morphologically indistinguishable from those seen in renal epithelial cells in human cases of nephrosis or nephritis. Although it is perhaps not permissible to apply results obtained in lower animals directly to human beings, the outcome of the experiments nevertheless strongly suggests that colloid droplets are the result of reabsorption and storage of protein from the tubular lumina after passing through the damaged glomerular filtering membrane. The tremendous enlargement, signs of degeneration and desquamation of tubular cells in cases of excessive protein storage indicate that the cells can be damaged during this procedure. Colloid droplets, therefore, are not the result of

cell degeneration but they can be the cause of degeneration of tubular epithelial cells.

By analogy, the morphologic changes in the kidneys in human lipoid nephrosis are due to an increased permeability of the glomerular capillaries and a high serum cholesterol. Bing and Starup [25] have shown that the excretion of cholesterol in renal disease runs parallel to the excretion of creatinin, urea and albumin. By increasing the permeability of the glomerular capillaries (uranium nitrate) with simultaneous experimental hypercholesteremia, Weltmann and Biach [26] and Richeri [27] produced lipoid changes in the renal tubular epithelial cells. Similarly, Korányi and Hámori [28, 29] showed again that injury of the glomerular capillaries makes the glomerulus permeable for serum albumin. It is this increased permeability of the glomerulus which is essential and primary in bringing about secondary morphologic tubular changes such as colloid droplets and lipoid droplets.

Conclusions

Colloid droplets in the lining cells of renal tubules of urodeles are due to absorption and storage of protein from the tubular lumina. "Natural" colloid droplets are not infrequently encountered in the "open" nephrons of urodeles as a result of the passage of protein-containing peritoneal fluid through the nephrostomes into the kidneys.

Intraperitoneal deposition of various protein-dye compounds, including homologous species proteins, is followed by the appearance of colored colloid droplets in the cells of the tubules of the "open" nephrons; their appearance was first observed 2 days after injection and they were still present 328 days afterwards.

In general, the size of the colloid droplets depends on the time elapsed since they were formed: at first they are large and occupy the supranuclear zone of the cytoplasm, but later they move towards the base of the cells where they become smaller.

No histologic evidence of splitting or digestion of colloid droplets within the renal epithelial cells could be detected. Partial reduction of colloid droplets is due either to desquamation of entire cells or to discharge of particles from tubular lining cells through tiny pores in the basement membrane of tubules, after which these are taken up by phagocytic stroma cells.

No specific site of storage within the tubules of proteins of various molecular sizes could be demonstrated; all proteins are absorbed and stored in the lining cells throughout the entire length of the "open" nephrons. Fats and lipids deposited in the peritoneal cavity of urodeles are phagocytized and stored in the tubules of the "open" nephrons in the same manner as proteins.

Protein-dye compounds injected intravenously into urodeles and mice partly pass through the glomeruli and are reabsorbed and stored by the lining cells of the renal tubules.

Parallels are drawn between the morphologic changes seen in human renal disease and those experimentally produced in urodeles.

NOTE: We wish to express our appreciation to Ruth Silbowitz for her technical assistance.

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DESCRIPTION OF PLATES

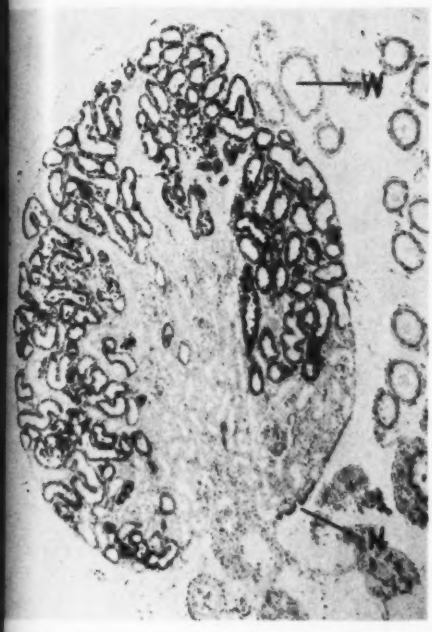
PLATE 161

FIG. 1. *Amblystoma punctatum*. Male. Four intraperitoneal injections, each of 0.5 cc. casein-R salt solution; 10 days after the first injection. The "open" nephrons are conspicuous due to the presence of casein-R salt in the tubular lining cells. N = nephrostome, W = wolffian duct. × 40.

FIG. 2. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. necturus serum albumin-R salt, 14 days after the first injection. The single segmental "open" nephron is clearly defined by the presence of albumin-R salt in the tubular lining cells. N = nephrostome, W = wolffian duct. × 15.

FIG. 3. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 20 days after the first injection. Large, pale colloid droplets are seen in the supranuclear zone, and small, dark granules are present in the basal portion of the lining cells of an "open" tubule. × 460.

FIG. 4. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. egg albumin-R salt, 18 days after the first injection. Segment of kidney cleared according to the method of Spalteholz. The "open" tubules are clearly defined throughout their entire length due to the presence of egg albumin-R salt within the tubular lining cells. Gl = lines parallel to midline where glomeruli are located, some of which are faintly visible. X = dye-protein in glomerular space regurgitated from the tubule. N = junction of nephrostomial canal and "open" tubule. × 10.



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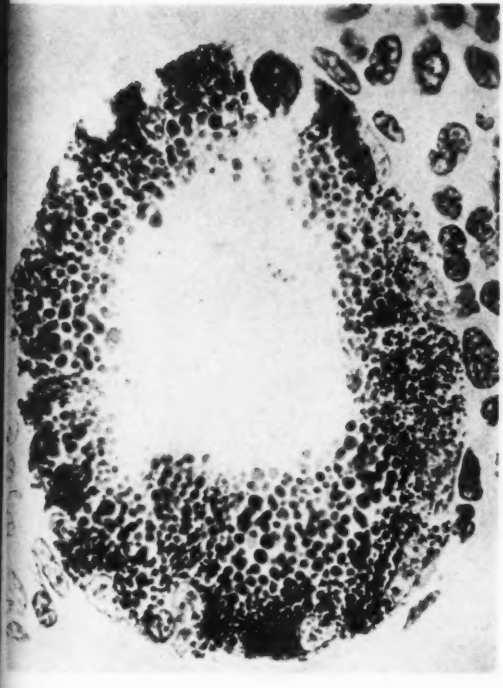

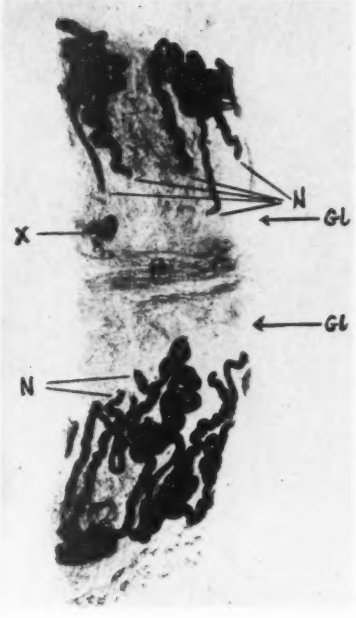

4

Smetana and Johnson

Origin of Colloid and Lipoid Droplets

PLATE 162

FIG. 5. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. necturus serum albumin-R salt, 60 days after the first injection. Colored granules and droplets are present in the basal portion of the lining cells of the "open" tubules; untinted "natural" colloid droplets occupy the supranuclear zone of the cytoplasm. × 460.

FIG. 6. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 20 days after the first injection. Distension of lining cells of "open" tubules by colloid droplets with rupture of cell membrane and desquamation of entire cells into the lumina of the tubules. × 230.

FIG. 7. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 87 days after the first injection. Dark red colloid droplets and granules are seen in the lining cells of the tubules and in phagocytes of the stroma outside the tubules. BM = basement membrane of "open" tubules. × 1050.

FIG. 8. *Necturus maculatus*. Male. Four intraperitoneal injections, each of 0.5 cc. dog serum globulin-R salt, 87 days after first injection. Extrusion of colored granules from a lining cell of the tubule through an opening in the basement membrane into the stroma where the material is taken up by a phagocyte. BM = basement membrane, P = pore in the basement membrane. × 1050.

Am

Sm

Fig. 5

Fig. 6

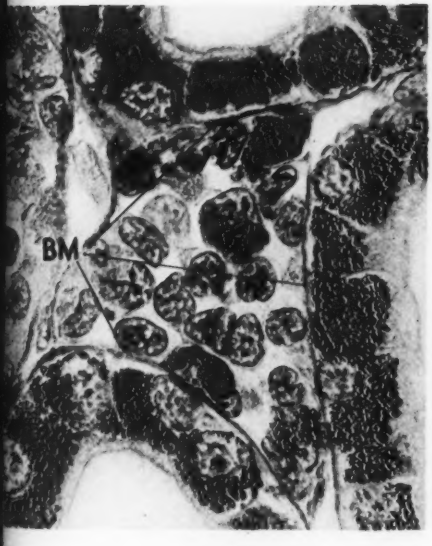

Fig. 7

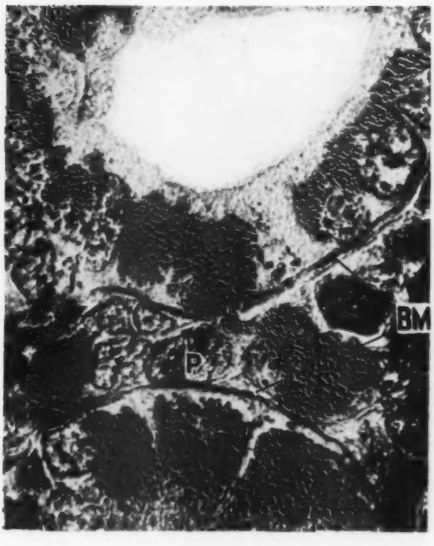

Fig. 8

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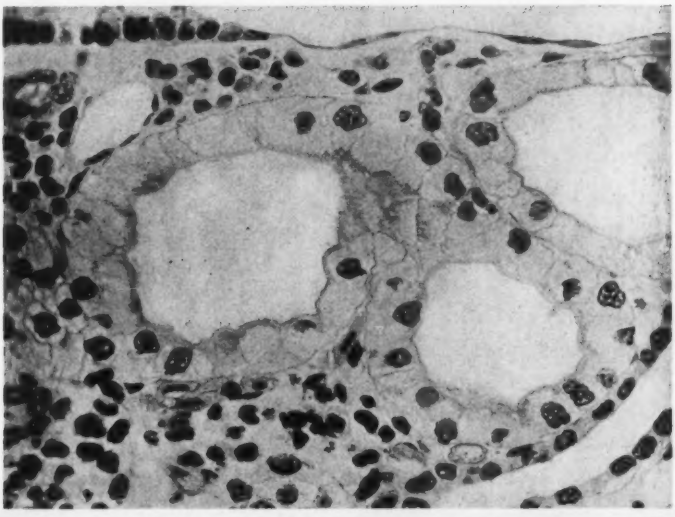
PLATE 163

FIG. 9. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Paraffin section stained by hematoxylin and eosin. Large, vacuolated lining cells of "open" tubules. A few small colloid droplets are seen along the inner border of the tubules. × 230.

FIG. 10. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Frozen section stained with scharlach R and hematoxylin. Lipoid-containing casts in lumina of "open" tubules and lipoid droplets in lining cells. × 230.

FIG. 11. *Necturus maculatus*. Male. Five intraperitoneal injections, each of 0.5 cc. of human serum, case of nephrosis, 35 days after the first injection. Frozen section stained with scharlach R and hematoxylin. Picture taken under the polarizing microscope. Doubly refractive cholesterol crystals in the tubular lining cells. × 230.



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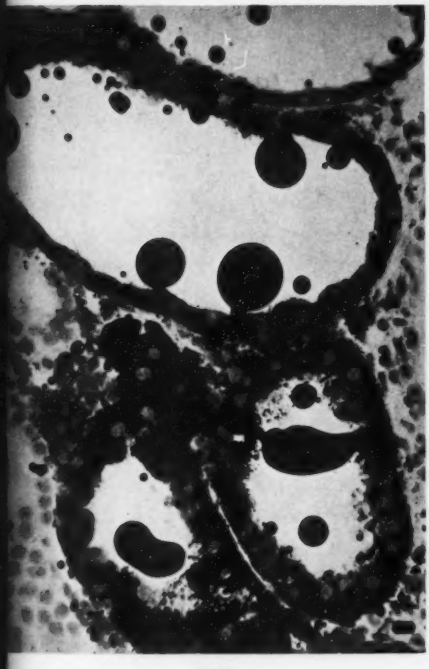

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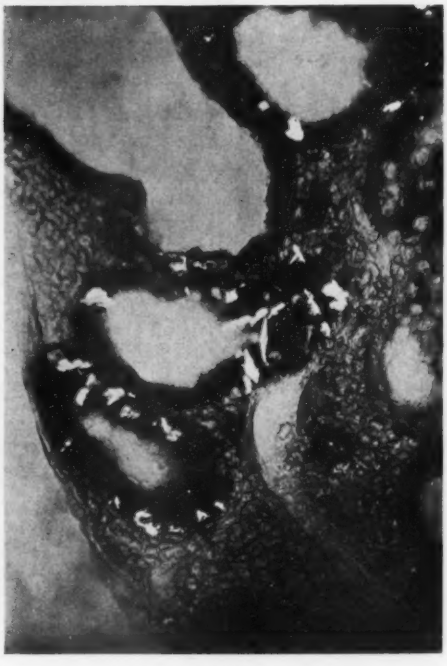

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Smetana and Johnson

Origin of Colloid and Lipoid Droplets

EFFECTS OF CENTRIFUGATION ON INTRANUCLEAR INCLUSIONS PRODUCED BY SUBCUTANEOUS INJECTIONS OF ALUMINUM OXIDE *

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By a program of work begun in 1935, we have sought to obtain information on the nature of intranuclear inclusions produced by viruses. The ultracentrifuge developed by Beams and Pickels [1, 2] has been used in all of these studies. It has been learned that there is a difference in relative specific gravity among inclusion bodies produced by various viruses. Herpes virus produces inclusions which are lighter than either the nucleoplasm or the basichromatin and oxychromatin.[3] Herpes is the only example of a granular type of inclusion body thus far investigated. However, several of the amorphous, nongranular types of inclusion bodies have been studied. In the submaxillary gland disease of guinea pigs the order from the lighter to the heavier pole of the centrifugated nucleus is nucleoplasm, inclusion body and a mixture of oxychromatin and basichromatin.[4, 5] In fox encephalitis, the sequence proves to be similar except that the inclusion body consists of two parts; an original basophilic center and a mantle of oxychromatin.[6]

The present investigation on intranuclear inclusions produced by chemicals represents further effort to identify and distinguish oxychromatin on the basis of its reaction when centrifugated, and when this has been accomplished to apply this information to acidophilic inclusions of virus origin.

Numerous investigators (Luger and Lauda,[7] Lee [8] and Blackman [9]) have produced an amorphous, nongranular type of intranuclear inclusion by introduction of various heavy metals, but in all of this work there is a possibility that the chemical used merely stimulated a latent virus which then made itself evident through the presence of an inclusion body. This alternate interpretation was removed, however, by the work of Olitsky and Harford [10, 11] when they tested, by passages and other means, the inclusions produced after subcutaneous injections of aluminum, iron, carbon and other substances. We employed their technic for injection of aluminum oxide and obtained similar intranuclear inclusions. Centrifugation of these inclusions has demonstrated a reaction similar to that of other amorphous, nongranular inclusions and has offered further evidence that this type of inclusion, when it

* Aided by a grant from the Research Council of Iowa State College. This aid supplemented facilities made available at the Veterinary Research Institute.
Received for publication, January 29, 1942.

accompanies a virus disease, probably is composed in part or entirely of oxychromatin.

Materials and Methods

Injections of aluminum oxide, aluminum hydroxide, ferric hydroxide and carbon were tried. The best results were obtained with aluminum oxide and all of the data recorded are based on use of this chemical. In the early attempts a 1 per cent suspension in saline solution was used, but this proved so destructive to the infiltrating cells that the lower limit which Olitsky and Harford [10] recommended, namely, 0.75 per cent, was used thereafter. Even this concentration, in our hands, produced more destructive effects than we expected to find after examination had been made of a slide which was sent to one of us (A.M.L.) several years ago by Dr. Olitsky.

Various subcutaneous areas were injected: back, side, abdomen, axilla and groin. Best results were obtained when the nodules were removed from 17 to 23 days after injection. Several stains were tried and the only one which gave good differentiation was phloxine-methylene blue, as recommended by Olitsky and Harford.[10] Azure-eosin proved unsatisfactory on this material.

The ultracentrifuge employed was constructed according to directions given by Beams and Pickels.[2] The air pressure varied from 93 to 120 pounds and the maximum speed as determined by a Galton whistle was 3400 revolutions per second. The range in centrifugal force was from about 300,000 to 550,000 times gravity. Half of the excised nodule was placed in the centrifuge and half in a dish of Locke's solution. When centrifugation was completed both pieces were fixed in Zenker's fluid with 5 per cent acetic acid added.

Observations

It has been emphasized in earlier publications that in order to analyze the action of a virus upon a cell, it is necessary to distinguish between the reaction of the nucleus to the presence of an inclusion body and that due to the generalized destructive action of the virus or foreign agent which leads to necrosis of the cell. These two reactions proceed independently and the only effect which the latter may have on the former is to cut short the sequence of nuclear reactions so that the full response to the aging of the inclusion body is not obtained.

The Process of Inclusion Formation

Early nuclear reactions were not abundant in our material; when observed, however, the distribution of chromatin and the structure of the linin network is quite similar to that found in the normal nucleus

except that masses of basichromatin aggregate into clumps around an acidophilic inclusion body (Fig. 1). Were other inclusion-bearing cells absent from the preparation, one would probably consider the central mass to represent a plasmosome nucleolus, surrounded by aggregated chromatin masses. The combined structure would be called an amphinucleolus.* With increasing age the small discrete basichromatin granules scattered over the linin network tend to aggregate into larger clumps and these move toward the nuclear membrane. In so doing they disappear from the linin network and the spaces of the network become larger. Chromatin granules still adhere to the surface of the acidophilic chromatin body (Fig. 2). In this particular nucleus the inclusion body was smaller than in the earlier stage; this confirms what has been emphasized in studies on virus-formed inclusions, that the size of the inclusion body is not necessarily a measure of its age, and that the degree of nuclear reaction is a more reliable criterion.[5, 6]

The process of margination continues, as well as coalescence of chromatin granules to form larger clumps. Frequently flecks of basichromatin still remain attached to the inclusion body after most of it has completely marginated, and ultimately all of it leaves the inclusion body. There still remain for a while, however, strands of the linin network, which extend across the halo surrounding the inclusion body (Fig. 3). The linin threads become less abundant and finally disappear, leaving an amorphous, nongranular inclusion body surrounded by a halo of nucleoplasm (Fig. 4). At such a stage of development the picture is morphologically identical with the nongranular type of inclusion body so frequently described in the literature as the sequela of a virus infection.

It has been difficult in these preparations to draw a sharp line between effects due to the formation of the inclusion body and the more generalized death sequence brought about by the chemical injected because the very conspicuous death reaction has frequently masked the other. In many nodules examined every nucleus of every type of cell present was a small pyknotic sphere. But on the basis of previous work it is suggested that the margination reaction is a response to the presence of the inclusion body and that the clumping of the chromatin is part of the sequence which may lead to death of the cell. Clumping in itself, however, does not predestine the death of the cell, for ample evidence has been offered that the chromatin of the nucleus may be contracted to a small, dense mass and then return to the normal dis-

* Olitsky and Harford[10] frequently use the term nucleolus. From the text it is evident that it is the karyosome or basichromatin type which is meant. They do not discuss the possible relationship of plasmosome nucleolus and inclusion body. They did note that amphinucleoli are not common, which agrees with our findings.

persed state without any apparent ill effect.[12, 13] However, when the nuclear membrane begins to shrink and there is a marked loss of nucleoplasm, what is probably an irreversible death change has set in and will lead to a pyknotic nucleus. Typical steps in the process are shown in Figures 5 to 8. During the process there seems to be very little fundamental change affecting the basichromatin or the inclusion body. It seems to be limited almost entirely to the loss of nucleoplasm, although, during the shrinking process, there is frequently observed a tinging of the nucleoplasm as if some of the chromatin had gone into solution.

Centrifugation of Inclusion-Bearing Cells

It is obvious that in the centrifugated nuclei the exact stage of nuclear reaction will be difficult to estimate because the basichromatin is concentrated during the process into a more or less compact mass at the centrifugal pole. However, one can break up the continuous process described in the preceding section into about three main divisions: early stage, adherence of basichromatin to the inclusion body; middle stage, absence of basichromatin on the inclusion body but no evidence of nuclear contraction (linin fibers may or may not extend from the inclusion body to the nuclear membrane); and last stage, loss of nucleoplasm and contraction of the nuclear membrane.

A few examples of the early stage have been found in the centrifugated preparations. They show abundant linin fibers stretched across the nucleus. The stage of nuclear reaction in Figure 9 is probably similar to that in the uncentrifugated nucleus shown in Figure 2. The next stage shows clearly the relative specific gravity of the three substances. The nucleoplasm is lightest, the inclusion next and the basichromatin is the heaviest (Figs. 10, 11, 12 and 14). There was no evidence of oxychromatin (other than that which forms the inclusion bodies) separating from basichromatin.

The last stage is represented by Figures 13, 15, 16 and 17. The nucleus in Figure 13, before centrifugation, probably corresponded quite closely to that shown in Figure 5 and illustrates an early stage in the shrinkage process. Loss of nuclear sap and shrinkage of the nuclear membrane does not change the relative specific gravity of the inclusion body and basichromatin (Fig. 15). Figures 16 and 17 were included because they show how the centrifugated nucleus may be turned at right angles when it strikes a barrier, in this case the cell wall of the enclosing giant cell.

The question naturally arises as to how much damage is being done to the cell by the process of centrifugation. Is the cell killed first and then the material stratified, or is the cell still alive at the end of the

process of centrifugation? Guyer and Claus [14, 15] have contributed information in regard to these questions by implanting tissues in which the chromatin had been thrown entirely to one pole and have found that the implant survived. Beams and King [16, 17] followed the development of Ascaris eggs while they were centrifuged at 150,000 times gravity for 4½ days. They conclude, ". . . . that the killing of cells by the present methods of centrifugation is usually due to mechanical distortion or disruption (prevented in Ascaris eggs by the presence of a very resistant shell) rather than to a disturbance of the spatial relationship of their molecular parts."

Our approach to this problem has been a cytological one. Some nodules were centrifugated for 2 hours instead of the usual hour, and in this material every nucleus was clearly dead (Figs. 18 to 20). The cytological appearance was so much in contrast to the usual appearance with which we have been dealing that it increased our confidence that the typical configurations after centrifugation were not death artefacts. The killed nuclei were homogenous with no indication of the usual nuclear structure or inclusion bodies and also no indication of stratification due to centrifugating (Fig. 20). Some nuclei which had not yet completely developed the full death picture showed some evidence of stratification (Fig. 19), and a very few were found which had the appearance shown in Fig. 18. This resembles a centrifugated cell which is still living, except that even here there was evidence of laking of chromatin and loss of staining affinity. It is evident from the few nuclei which showed centrifugation effects that probably all of them had had this appearance before they were killed. Therefore, following death, the liquefied (?) contents became uniformly dispersed throughout the nucleus. It is not known whether the dispersion takes place while centrifugation is in progress or in the minute or less after centrifugation, before the fixative penetrates the cells.

Discussion

Are inclusion bodies produced by aluminum oxide and other metals identical with the amorphous or dropletlike inclusion bodies associated with virus diseases? Olitsky and Harford [10, 11] are of the opinion that they are identical and our own work confirms this opinion. The acceptance of this opinion forms a reference point against which previous conclusions based on centrifugation studies may be tested. There is little doubt in the case of aluminum oxide stimulation that the inclusion body is derived from the oxychromatin which is a normal constituent of the nucleus. It can be traced back to what would generally be regarded as a hypertrophied plasmosome and then by a gradual loss of

adherent basichromatin, which moves to the margin, it becomes an inclusion body identical with the amorphous type of virus origin. Oxychromatin has a lower specific gravity than that of basichromatin, which fact was noticed first in the noninclusion-bearing cells of the fox. This agrees with results of the present study, and just as the amorphous inclusions produced by aluminum oxide come to lie on the centripetal side of the basichromatin so do the inclusions of submaxillary gland disease of the guinea pigs and of fox encephalitis stratify to similar positions. It is true that in these two virus diseases the inclusion bodies could not be traced back to a plasmosome nucleolus; in fact when one was present it was pushed to one side and marginated with the karyosome nucleolus which surrounded it. Nevertheless, in fox encephalitis the acidophilic material of the inclusion body arose by a generalized separation from basichromatin. Another point of similarity between amorphous inclusions of nonvirus and virus origin is their union with linin fibers in early stages of development and their separation later. A point of difference between the two is the fact that chromatin adheres to the inclusion (plasmosome nucleolus) of nonvirus origin when it is in its early stage of development, whereas in fox encephalitis and submaxillary gland disease it returns to join the oxychromatin inclusion body only in its late stages. This may prove to have more significance than it seems to have at the present time. In both, however, centrifugation fails to separate basichromatin when it is adhering to an inclusion body.

In the program of investigation thus far, the "granular type" of inclusion (herpes simplex in the rabbit cornea) has proved to be definitely different from the amorphous type. It would be especially interesting to learn whether pseudorabies virus and B-virus, when centrifugated, would react as does that of herpes. Also it would be helpful to know if a "particulate type" of virus inclusion (yellow fever) would behave like an amorphous or a granular type. It would probably help to answer the question whether the particulate type of inclusion is really oxychromatic degeneration as described in numerous publications.*

Summary and Conclusions

1. Experiments described by Olitsky and Harford on the production of intranuclear inclusions by subcutaneous injection of aluminum oxide have been repeated and their results confirmed.

2. The affected cells have been arranged in what is considered the

* Inclusions produced by pseudorabies, B-virus and panleukopenia of cats have been centrifugated since this paper went to press. It is evident from experiments thus far available that the type of tissue has an effect upon behavior of the inclusions during centrifugation.

probable sequence in the development of inclusion bodies and in the reaction of the nucleus which accompanies it.

(a) The earliest stage is the appearance of an amphinucleolus. The plasmosome portion becomes the future inclusion body.

(b) The chromatin, which in early stages was adherent to the inclusion body (plasmosome), leaves it and moves to the margin. Across the resulting halo are strands of the linin network.

(c) These break down and the inclusion body assumes an appearance identical with the amorphous or dropletlike inclusion body characteristic of some virus diseases.

(d) The last stage is necrosis which is made evident by shrinkage of the nuclear membrane and loss of nucleoplasm until all that remains is inclusion body surrounded by basichromatin and a nuclear membrane.

3. Centrifugation of these stages leads in each case to the same conclusion; namely, that the nuclear sap is the lightest substance, that the inclusion body is heavier and that the basichromatin is heaviest.

4. Comparison of these results with those from other centrifugation and cytological studies leads to the conclusion that oxychromatin is the material from which the acidophilic portions of the amorphous type of inclusion body, such as that found in submaxillary gland disease of the guinea pig and in fox encephalitis as well as those produced by heavy metals, are derived.

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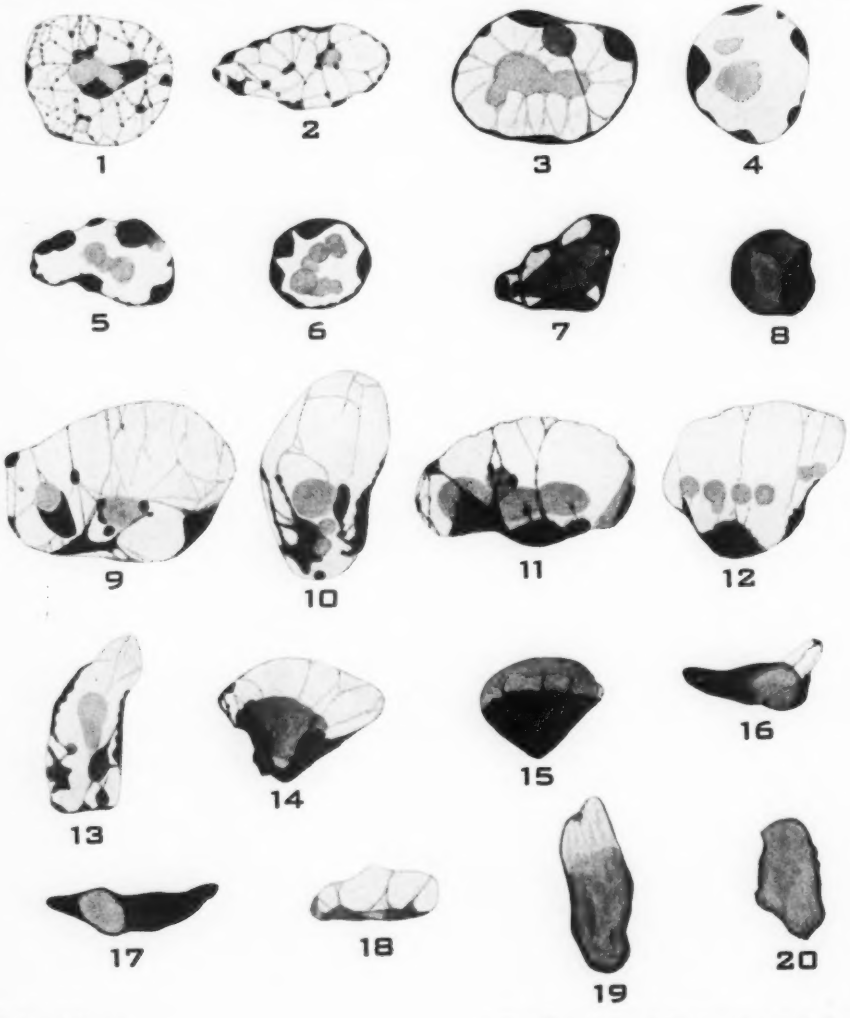
DESCRIPTION OF PLATE

PLATE 164

All nuclei were drawn with a camera lucida at an optical magnification of 2000x and a projection magnification of 3200x. There has been no reduction in reproduction. All figures are of nuclei of cells which infiltrated nodules resulting from the subcutaneous injection of aluminum oxide in the guinea pig. All nuclei were stained with phloxine-methylene blue. Nuclei in Figures 1 to 8 were not centrifugated. Nuclei in Figures 9 to 20 were centrifugated at forces which varied during the centrifugation process from 300,000 to 550,000 times gravity. Those in Figures 9 to 17 were centrifugated for 1 hour and those in Figures 18 to 20, for 2 hours. All drawings of centrifugated nuclei are oriented so that the centrifugal pole is directed toward the bottom of the plate.

For description of the individual nuclei the reader is referred to the text.



Birch and Lucas

Centrifugation and Intranuclear Inclusions

PATHOLOGIC ALTERATIONS IN SURFACE RELATIONSHIPS AND MORPHOLOGY AT THE HUMAN SYNAPSE *

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INTRODUCTION

Anatomically, the term "synapse" refers to the contiguous components in a succession of neurons. From the fact that the transfer of an impulse across the synapse is unidirectional, the structure may be thought of as having an afferent, or distributing, component and an efferent, or receptive, component. The receptive structure includes the cell body, its dendritic processes and the root of the axon. In many locations in the central nervous system all three of these cellular areas act in a receptive capacity (prominent in Purkinje cells of the cerebellar cortex and in the lateral groups of final-common-path cells of the spinal cord). In other locations (nucleus dorsalis) the cell body and the root of its axon appear to be the principal receptive structures, with the dendrites playing little part. In still other regions (substantia gelatinosa Rolandi) the dendrites are described as the only receptive elements. The afferent or distributing component in each case includes the fibrillar axonic ramifications and the terminals (boutons) which bear on the receptive structures in question. The complexity of the afferent limb of the synapse appears to vary with the functional attributes of the efferent limb. Probably a great many distinct sources exist for supplying bouton connections to the final-common-path cells of the spinal cord. On the other hand, in some internuncial chains, the conterminous parts may represent relatively linear successions.

That these synaptic relationships may be disturbed under pathologic or experimental influence seems probable, although the demonstration of such changes has been subject to question. The use of morphologic or numerical changes in peripheral nerve endings as acceptable criteria of pathologic or experimental influence on the associated nerve cells or fibers is well established (Jackson,[1] Cajal,[2] Lawrentjew,[3] Gibson,[4] Carey,[5] Speidel[6]). Although one might reasonably expect similar accord with regard to central synaptic effects, nothing of the sort has appeared. Since Hoff's introduction of the bouton method as a means of establishing tract-nuclear relationships, a great many controversial reports have been presented (Hoff,[7, 8] and Barnard[9]). Probably the

* Read in part before the American Association of Neuropathologists, Atlantic City, N.J., June 9, 1941.
Received for publication, March 9, 1942.

most general criticisms of the acceptability of the procedure have been on the bases of (1) technic and (2) inadequacy of control. It must be admitted that these criticisms are most applicable to the human material which is employed in the present study. Nevertheless, in view of the current interest in central synaptic phenomena, it has seemed permissible to proceed by anticipating the criticisms as far as possible with quantitative methods which establish a predictable synaptic morphology with the standard technic used.

In a recent report [10] the boutons (nerve terminals) of the human spinal cord, appearing in block silver preparations, are described in five primary morphologic groups (types)—small loops, large loops, fila-

TEXT-FIGURE 1. This schematic diagram of a hemisection of the gray matter of the thoracic cord shows the average percentages of granular forms (blackened sectors) of terminals ending in relationship to the cells of the principal nuclear groups in nonneuropathologic cases under 30 years of age. The posterior sector in the gray matter represents the boutons around cells of the sensory and internuncial groups; the medial sector, of the nucleus dorsalis; the lateral intermediate sector, of the intermediolateral column; and the anterior sector, of the somatic efferent columns.

TEXT-FIGURE 2. A diagram corresponding to Text-Figure 1, but for nonneuropathologic cases over 30 years of age.

TEXT-FIGURE 3. The percentages of granular forms of terminals around cells of the principal nuclear columns in a thoracic field of case H108, poliomyelitis.

mented loops, fibrillated bulbs, and opaque or granular masses—and three subgroups (forms)—regular, thickened, and granular. A schema of this classification is presented as the legend to Figures 1, 2 and 3 with references in appropriate columns to the morphologic "types" and "forms" of boutons appearing in the photomicrographs. Because of the relative unimportance (numerically) of regular and thickened filamented loops, the publication of additional pictures to illustrate these structures seems unwarranted. A quantitative study [11] of the nerve terminals in 26 cases with no clinically demonstrable pathology of the central nervous system has revealed that the same proportions of the various morphologic types of boutons occur from case to case regard-

less of age (after 1.5 years), provided certain limitations in the technic are observed and the same regions, including similar cell groups, are compared. The percentages of regular, thickened and granular boutons (forms), however, vary greatly from case to case and from cell column to cell column in the same material. In general, granular forms occur in relatively greater number in persons of middle age or older. It has been shown further that technic and autolysis are of little importance in accounting for the demonstrable variations in human bouton morphology.

Of the influences of recognized potency in causing damage to the central nervous system which are operative in most cases that come to autopsy, the following have been mentioned: arteriosclerosis, inanition, intoxication, fever, dehydration, systemic infection and additional agonal correlatives. The material examined in the present study has been subject, as well, to lesions of the central nervous system which were clinically manifest. The immediate problems in connection with this material are (1) the determination of the manner in which, and the extent to which, surface relationships at the human synapse might be disturbed in neuropathologic involvements, and (2) the identification of a morphologic index of pathologic influence. An understanding of both numerical and morphologic variables is considered essential to the interpretation of findings in specific cases to be described. The observations on the neuropathologic material employed in the present study will be considered in the following sequence: (1) surface area of the receptive component of the synapse; (2) number of terminals and contact surface at the synapse; (3) alteration in number of terminals (related to pathology, regional differences, autolysis, technic, age); (4) alteration in morphology of terminals; (5) influence of the fundamental nature of the pathologic process on both number and morphology of terminals; (6) influence of duration of the pathologic process; (7) the factor of distance between the lesion and the alterations observed; (8) progressive and retrograde effects at the synapse; (9) individual case data. Since the pathologic changes of the receptive component of the synapse (chromatolysis, fragmentation, shrinkage, nuclear change) are fairly well established, no special effort has been made to review this field. The observations in the present study are primarily on the distributing components of the synapse, and, of these, particularly on the nerve terminals, or boutons.

Method and Material

The spinal cords used in the present study were removed at autopsy by the Kernohan [12] method and the sections treated with Cajal's silver

TABLE I
Essential Data from Case Histories

Case	Age	Autolysis time	Formalin time	Duration of lesion	Diagnosis
H1	45 yrs.	4 hrs.	25 hrs.	29 da.	Fracture at 4–5 cervical vertebrae
H3	65 yrs.	2 hrs.	26 hrs.	2 yrs.	Hodgkin's disease; generalized sepsis; old pontine infarct
H5	12 yrs.	3 hrs.	15 hrs.	6 da.	Toxic encephalomyelitis (Landry)
H6	58 yrs.	24 hrs.	19 hrs.	2 yrs.	Cerebral thrombosis with old encephalomalacia
H7	15 mos.	8 hrs.	70 hrs.	15 da.	Purulent meningitis
H8	41 yrs.	17 hrs.	22 hrs.	5 wks.	Left cerebral tumor
H9	57 yrs.	11 hrs.	21 hrs.	9 da.	Carcinoma of esophagus (inanition)
H11	61 yrs.	3 hrs.	14 hrs.	3 da.	Arteriosclerosis; suppurative pericarditis; uremia
H12	73 yrs.	7 hrs.	24 hrs.	4 da.	Cerebral hemorrhage
H16	36 yrs.	13 hrs.	10½ mos.	26 yrs.	Amputation of right leg (amyloidosis; uremia)
H17	39 yrs.	7 hrs.	22 hrs.	4 mos.	Metastatic brain tumor
H18	54 yrs.	13 hrs.	23 hrs.		Cerebral arteriosclerosis
H20	57 yrs.	6 hrs.	23 hrs.		Luetic meningitis
H23	70 yrs.	2½ hrs.	22 hrs.		Cerebral arteriosclerosis
H24	57 yrs.	13 hrs.	22 hrs.		Carcinoma of stomach (inanition)
H27	68 yrs.	11 hrs.	26 hrs.		Luetic meningitis
H29	52 yrs.	11 hrs.	23 hrs.	5 yrs.	Left cerebral tumor
H31	73 yrs.	5 hrs.	20 hrs.		Perforating duodenal ulcer; arteriosclerosis
H37	20 yrs.	2 hrs.	24 hrs.	2½ mos.	Brain tumor obstructing ventricles
H39	72 yrs.	7 hrs.	23 hrs.	5 mos.	Left leg amputation; diabetes mellitus; arteriosclerosis
H40	53 yrs.	15 hrs.			Diabetes; hypoglycemia
H41	27 yrs.	3 hrs.	17 da.	7 da.	Polioencephalomyelitis
H42	2½ yrs.	4 hrs.	28 hrs.	4 mos.	Tumor of right cerebellar hemisphere
H43	4½ yrs.	11 hrs.	21 hrs.	3 wks.	Postinfectious encephalomyelitis
H44	62 yrs.	4 hrs.			Diabetes; carcinoma of stomach
H45	51 yrs.	4 hrs.	39 hrs.	3 mos.	Right brain tumor (glioblastoma multiforme)
H47	23 yrs.	14 hrs.	39 hrs.	2 yrs.	Uremia; chronic glomerulonephritis; otitis media
H48	47 yrs.	13 hrs.		6 da.	Tuberculous pneumonia; chronic alcoholism
H49	41 yrs.	3 hrs.	13 hrs.	3 mos.	Metastatic brain tumor; old cord demyelination
H50	38 yrs.	5 hrs.	26 hrs.	1½ yrs.	Multiple myeloma with destruction of cord below T_7
H51	16 yrs.	4 hrs.	40 hrs.	3 mos.	Seminoma of left testicle; uremia
H53	14 yrs.	12 hrs.	30 hrs.	37 da.	Purulent meningitis
H55	80 yrs.	3 hrs.			Cerebral arteriosclerosis
H56	54 yrs.	3 hrs.	32 hrs.	1½ mos.	Hemolytic streptococcic septicemia
H58	18 yrs.	3 hrs.	57 hrs.		Appendectomy followed by hyperpyrexia; pituitary dwarfism
H60	60 yrs.	14 hrs.	23 hrs.	13 da.	Tetanus
H62	53 yrs.	2 hrs.	24 hrs.	1 yr.	Right cerebral tumor
H64	79 yrs.	5 hrs.	30 hrs.	2 yrs.	Metastatic vertebral tumor (L_2–L_5) with damage to cauda
H70	79 yrs.	5 hrs.	20 hrs.	73 yrs.	Pneumonia; old poliomyelitis
H71	29 yrs.	3 hrs.	48 hrs.		Generalized miliary tuberculosis; tuberculous meningitis
H72	33 yrs.	3 hrs.	7 da.	1½ mos.	Left frontal brain abscess
H73	65 yrs.	8 hrs.	14 da.	3 wks.	Multiple sclerosis (?); old toxic myelitis(?); pernicious anemia(?)

TABLE I—*Continued*

Case	Age	Autolysis time	Formalin time	Duration of lesion	Diagnosis
H74	3 mos.	12 hrs.	20 hrs.		Hydranencephalus
H75	28 yrs.	24 hrs.	18 hrs.	1 da.	Fracture of cervical vertebrae
H76	67 yrs.	2 hrs.	22 hrs.	1 da.	Fracture, T9 and T10
H77	70 yrs.	16 hrs.	22 hrs.		Cerebral arteriosclerosis
H81	75 yrs.	3 hrs.	21 da.		Cerebral arteriosclerosis
H82	20 yrs.	3 hrs.	20 hrs.	10 da.	Subacute bacterial endocarditis; clinical encephalitis
H83	62 yrs.	5 hrs.	15 mos.	7 wks.	Fracture of 5th cervical vertebra
H84	52 yrs.	13 hrs.	15 mos.	3 wks.	Purulent meningitis
H85	15 yrs.	3 hrs.	16 mos.	3 wks.	Purulent meningitis
H86	45 yrs.	7 hrs.	19 mos.	7 da.	Fracture and fat embolism
H87	61 yrs.	27 hrs.	19 mos.		Luetic meningeoencephalitis (general paresis with tabes)
H88	20 mos.	3 hrs.	6 yrs.		Avitaminosis A; necrosis of brain and cord
H89	39 yrs.	24 hrs.	13 yrs.	10 mos.	Actinomycotic myelitis
H90			5 yrs.	2 da.	Poliomyelitis
H91	14 yrs.	2 hrs.	6 yrs.	7 da.	Fracture of cervical vertebrae
H92	55 yrs.		12 yrs.		Subacute necrotic myelitis
H93	28 yrs.	4 hrs.	4 yrs.	7 da.	Purulent meningitis
H94	23 yrs.	2 hrs.	5 yrs.	12 da.	Poliomyelitis
H95	10 yrs.	2 hrs.	5 yrs.	3 da.	Polioencephalomyelitis
H96	46 yrs.	7 hrs.	8 yrs.	7 wks.	Vitamin D deficiency(?); degeneration of anterior horn cells
H97	36 yrs.	2 hrs.	7 yrs.		Malnutrition
H98	30 yrs.	2 hrs.	10 yrs.	30 da.	Meningeoencephalomyelitis
H99	28 yrs.	3 hrs.	10 yrs.	27 yrs.	Diaphragmatic hernia; old poliomyelitis
H100	28 yrs.	5 hrs.	12 yrs.	16 da.	Cord necrosis
H101	25 yrs.	2 hrs.	6 yrs.	5 da.	Meningeoencephalomyelitis (poliomyelitis)
H102	39 yrs.	2 hrs.	15 yrs.	1 mo.	Lues; resolving purulent meningitis
H103	37 yrs.	10 hrs.	11 yrs.	28 da.	Cord necrosis
H104	48 yrs.	9 hrs.	5 yrs.		Pigment degeneration and atrophy of ganglion cells
H105	54 yrs.	2 hrs.	17 yrs.		Pellagra; clinical encephalitis
H106	14 yrs.	9 hrs.	10 yrs.	5 da.	Poliomyelitis with involvement of dorsal root ganglion cells
H107	10 mos.	2 hrs.	10 yrs.	4 da.	Hemorrhagic encephalomyelitis
H108	16 mos.	6 hrs.	5 yrs.	12 da.	Poliomyelitis
H109	8 yrs.	2 hrs.	5 yrs.	2 wks.	Postmeasles encephalomyelitis
H110	22 yrs.	3 hrs.	6 yrs.	3 da.	Polioencephalomyelitis
H111	52 yrs.	3 hrs.	6 yrs.	50 yrs.	Ulcerative colitis; peritonitis; ancient poliomyelitis
H112	9 yrs.	6 hrs.	12 da.	7 da.	Tetanus
H113	49 yrs.	12 hrs.	6 yrs.	? yrs.	General peritonitis; cerebellar atrophy
H114	59 yrs.		9 yrs.	1 yr.	Cerebellar atrophy
H115	74 yrs.		3 yrs.	18 yrs.	Cerebellar atrophy

nitrate, modification 4 (after Barr [13]). Since the earlier reports, considerable material has been used which has lain in formaldehyde from 1 to 17 years. Although unsatisfactory in a few cases, some excellent preparations have been obtained from material fixed in formaldehyde for long periods. In checking the nature and distribution of lesions, routine methods have been employed, including hematoxylin and eosin,

cresyl violet, Marchi, Weigert, and Masson's trichrome stains. The boutons in certain locations, particularly in the cerebellar cortex, did not respond well to modification 4 and required staining with pyridine silver. In these instances the control blocks were handled in the same manner. Pyridine silver was also employed in the material from individuals under the age of 1 year with more success than attended the use of the usual method.

The method of counting and classifying the boutons and of determining their proportionate distribution has been described.[10] Where the procedure for cord nuclei was not applicable, as in cerebellar cortex and nuclei, methods were employed which will be described with the bouton distribution in the region. The procedures and methods of control will be explained in the treatment of each subdivision of the general observations.

The 81 cases examined in the present study are listed in Table I, together with essential data on prominent factors which might be expected to influence the bouton picture. It will be noted that the duration of the neuropathologic process could not be ascertained in many cases. In some of these the reason will be evident in the diagnosis, in others no information was available. It should be noted, too, that the duration of the neuropathologic processes does not necessarily correspond to the duration of the terminal illness, which in itself may be of considerable importance.

Observations

I. Surface area of the receptive component of the synapse

Preliminary to an inspection of altered conditions at the synapse it has seemed advisable to ascertain as closely as possible the normal surface relationships between the contiguous parts. In estimating the surface area of the receptive components of the synapse the following procedure has been employed: sections 10 μ in thickness were examined systematically under oil immersion, noting the number, linear dimensions, configuration and source of the nerve cell fragments in the various columns of the gray matter. The total cell surface area in each nuclear group in the section was computed by adding the surfaces of the cell fragments (computed individually by appropriate formulae fitting cones, spheres, cylinders, ellipsoids, or parts of each, depending on the configuration of the fragment in evidence). A linear estimate of total cell *body* length in the section of each column was computed as the product of the number of cell bodies cut times the depth of the section. This value divided by the average length of the cells of the location in question was taken as the theoretical number of cells cut

and distributed in slices in the section examined. The total surface area appearing in the region, divided by the theoretical number of cells, gave an estimate of the surface area per cell. In measuring, peripheral dendrite, dendrite root, cell body and axon root fragments were differentiated. (No fragment under 2 μ in diameter was counted since no synaptic contacts on cell structures of less than this magnitude have been noted by me.) The estimates of cell surface area and the proportion of the total area contributed by cell processes in the various columns of the spinal cord are given in the first two columns of Table II.

The differences in cellular surface area among the various columns

TABLE II

Contact Surface Area and Number of Boutons per Cell

Nuclear column	Computed cell surface (μ^2)	Process surface as % of total	Computed boutons per cell	Contact surface (μ^2)	Per cent of cell surface engaged in contact
Dorsal sensory and internuncial	756	35%	97	321	
Intermediate sensory and internuncial	1,053	44%	152	515	55.03%
Ventral sensory and internuncial	510	27%	116	376	
Nucleus dorsalis	4,372	52%	348	1,354	30.97%
Intermediolateral	1,563	38%	122	397	25.40%
Posteromedian	2,063	56%	362	1,410	
Anteromedian	3,949	63%	675	2,637	48.50%
Anterolateral	20,143	81%	1,624	6,349	
Posterolateral	56,479	81%	3,990	15,593	

(510 μ^2 to 56,479 μ^2) appear to be far greater than would be imagined from inspection of cross section area of the cell bodies. It is interesting to note the enormous relative surface area (81 per cent) contributed by the processes of the lateral groups of final-common-path cells. The cell body area listed for cells of the nucleus dorsalis included the surface of the root of the axon which in this case amounted to 30 per cent of the total surface.

Lassek [14] has recently cited three conceptions of the significance of cell caliber in the motor cortex: (1) that cell size varies with the "size of the movement" it governs (Jackson [15]); (2) that cell size varies with the distance over which its impulse must travel (Lewis [16]), and (3) that cell size depends on the extent of the unit it supplies (Fulton [17]). That these factors apply as well to the cells of the spinal cord would seem evident from the known distributions of the efferent groups and the nucleus dorsalis. On the same basis the axon connections of the receptive and internuncial groups would probably be somewhat as follows: ventral group to efferent cells of the same side and possibly

short homolateral intersegmental connections; intermediate group, long crossed and long homolateral intersegmental connections; and the dorsal group, shorter crossed and short homolateral intersegmental connections. The disproportion in cell size relative to its metabolic responsibilities, as evident in the surface area data of Table II, would suggest that additional factors are operative in dictating cell size. Of these, the provision of adequate receptive contact surface for varying complexities of afferent synaptic components would seem to be of prime importance. A comparison of the surface area on a cell of the nucleus dorsalis with that of a cell of the anterolateral group illustrates the point. The cell diameters in the two groups are approximately the same and the axons presumably travel about the same distance; but the surface area of a cell of the latter group is approximately four times that of a cell of the nucleus dorsalis (Table II). This suggests that cell size (surface) is perhaps more closely related to its receptive capacity than to its axon distribution.

A prominent exception to any of the above views relative to cell size is found in the constituents of the nucleus substantia gelatinosa Rolandi which are commonly described as second order neurons in the pain and temperature pathway. Their axons presumably pass to the opposite side and ascend in the spinothalamic tract—a fact which is entirely out of keeping with the size of these cells. It would seem necessary to view these cells, on the basis of size, either as very short internuncial elements or as unassigned by function. As reported previously, the synaptic relationships of these cells are obscure with the technics used in the present study.

2. NUMBER OF TERMINALS AND CONTACT SURFACE AT THE SYNAPSE

Estimates of the number of boutons ending in relationship to nerve cells (computations based on spherical shape of cell bodies) have been reported (Barr,[13] Minckler [10]). The present consideration is an effort to refine these estimates by basing the computation on more accurate determinations of cell surface [18] (Table II). The values listed in Table II (column 3) have been determined from the number of terminals counted on cell fragments, the average size of each, the optimal density of boutons on each part of the receptive component and the fraction of the available contact surface involved. These data are summarized in the last column as contact surface with an estimate of the percentage of the cell surface engaged in synaptic contact in the four main groups of cells (afferent and internuncial, nucleus dorsalis, intermediolateral column, and somatic efferent). Because of the selection of optimal preparations in determining bouton density on cell bodies, these data

should probably be considered maximal rather than average for the technic used (comparison may be made with actual counts representing number per cell body in a previous report [10]).

Crude estimates of total synaptic surface relationships in the human cord are given in Table III. The estimated number of cells in each column is a linear multiple (based on length of column) of the average theoretical number of cells appearing in each section counted. The total receptive surface is computed from the surface-per-cell value in Table II. The computed total values for boutons and for contact surface are likewise based on the data of Table II.

TABLE III
Estimated Total Synaptic Surface Relationships in Cord

Nuclear column	Estimated no. of cells	Estimated total cell surface ($mm.^2$)	Total no. of boutons	Contact surface ($mm.^2$)	Per cent of total by groups
Dorsal sensory and internuncial	1,820,000	1,375	176,540,000	646	
Intermediate sensory and internuncial	2,230,000	2,348	338,960,000	1,291	35.5
Ventral sensory and internuncial	1,390,000	708	161,240,000	389	
Nucleus dorsalis	188,600	825	65,632,800	247	3.8
Intermediolateral	1,023,000	1,599	124,806,000	399	6.1
Posteromedian	825,000	1,702	298,650,000	817	
Anteromedian	519,000	2,050	350,325,000	984	54.6
Anterolateral	72,000	1,451	116,928,000	696	
Posterolateral	40,000	2,260	159,600,000	1,084	
Total	8,107,600	14,318	1,792,681,800	6,553	

Assuming a measure of reliability for the data of Table III, it is interesting to note that approximately 18 per cent of the cells in all groups listed (those of somatic efferent groups) account for over 50 per cent of the total synaptic contact surface in the cord. It will be noted that a number of cord cell columns are omitted in Table III; these would, of course, increase the total values listed. The identification of the sources of terminals contributing to these synapses and a quantitative expression of the relative importance of these sources are problems of great importance in understanding spinal synaptic activity.

The remainder of the present paper outlines the procedures used in employing pathologic material in determining these relationships, based on alteration in number or morphology of afferent synaptic elements.

3. ALTERATION IN THE NUMBER OF TERMINALS AT THE SYNAPSE

Since errors in direct counts are magnified by transcribing the sample counts to a total synapse picture (as in Table III), it has seemed ad-

TABLE IV

Average Number of Boutons per Cell Section Appearing in the Various Nuclear Columns in Nonneuropathologic and Neuropathologic Cases

	Afferent and internuncial			Nucleus dorsalis			Intermediolateral			Somatic efferent			Total averages		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
Nonneuropathologic (19 cases)	899	1947	2.17	329	1320	4.01	192	440	2.29	850	4388	5.16	2270	8095	3.57
Neuropathologic (78 cases)	3745	10033	2.68	1044	4820	4.26	840	1690	2.01	4071	24117	5.92	9700	40660	4.19

visable to leave the values as expressions of the actual observations (number of terminals per cell section) in the comparisons below. Since the main influence to be examined as instrumental in effecting change at the synapse was pathologic involvement of nerve elements, a general comparison of the average number of terminals per cell section found in neuropathologic and nonneuropathologic cords was the first consideration (Table IV). With the exception of the terminals adjacent to cells of the intermediolateral column, it would seem from Table IV that the number of terminals was increased under the influence of disease. These average values are subject to a great many controllable variables and depend primarily, as will be shown later, on selection of case types. A more descriptive picture of this comparison is the graph (Table V) of the average number of terminals per cell section in all nuclear groups. From this table it is evident that the averages in the two groups correspond fairly well but that the extremes (cases showing less than two or more than seven boutons per cell section) are peculiar for the most part to the neuropathologic group. This greater variability in each direction for neuropathologic cases persists in the number of terminals in individual nuclear columns.

In arranging Tables VI, VII, VIII and IX an effort was made to ascertain to what extent this demonstrated variability in bouton counts may be related to regional differences in the spinal cord, to autolytic changes, to differences in technic and to age. The cases in each comparison have been selected to eliminate, as far as possible, all variables except the one in question. In Table VI the number of

terminals per cell section (averages) and the range in the counts for the number of boutons and cell sections indicated are given for nine sections at four levels. These counts were from cases H6, H23, H31, H12 of the present series, and nos. 41, 42, 52, 56, 59 of the series previously reported.[11] These patients varied in age from 58 to 83 years; in time between death and autopsy, from 3 to 24 hours; in time in formalin from 19 to 33 hours. Arteriosclerosis was common to all. The suggestions from the table that the internuncial cells are relatively

TABLE V

Distribution of Counts on 19 Nonneuropathologic Sections (o) and 92 Neuropathologic Sections (x) According to the Average Number of Terminals per Cell Section. Age Groups and Nuclear Columns are Not Differentiated

Number of boutons per cell section	
0–1	x x x x x x x x x o
1–2	x x x x x x x o
2–3	x x x x x x x x x x x x x x o o o o o
3–4	x x x x x x x x x x x x x x x x x x o o o o o
4–5	x x x x x x x x x x x x x x x x x x x o o o o o
5–6	x x x x x x x x x x x x o
6–7	x x x x x x x x o
7–8	x x
8–9	x x
9–10	x x
10+	x x

more receptive in the cervical enlargement and that the somatic efferent groups of the lower part of the cord have relatively more associations may be unfounded because of the small sample necessitated by selection of parallel cases.

Table VII compares the average number of boutons per cell section in two cases (H6, H18), autopsied 13 and 24 hours after death, with three cases (H23, H12, H3), autopsied 2, 2.5 and 7 hours after death. All were patients with arteriosclerosis, varying in age from 54 to 73 years, and all sections listed were from the thoracic region. The time in formalin varied from 19 to 26 hours. It seems apparent that no

TABLE VI
Comparison of Number of Boutons per Cell Section in Cord Regions

	Sensory and internuncial				Nucleus dorsalis				Intermediolateral				Somatic efferent			
	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range	Cell sections	Boutons	Av. no. per cell section	Range
Cervical (1 section)	49	94	1.92	1.5 to 2.9									55	327	5.95	3.0 to 8.7
Cervical enlargement (1 section)	71	217	3.06	2.4 to 3.8									100	541	5.41	4.3 to 6.6
Thoracic (4 sections)	144	396	2.75	1.6 to 3.7	82	482	5.88	4.6 to 7.0	59	168	2.85	2.5 to 3.0	178	1130	6.35	5.4 to 7.0
Lumbar enlargement (3 sections)	241	668	2.52	1.0 to 4.5	51	275	5.40	2.7 to 7.6	19	40	2.11	2.1 to 2.2	159	1171	7.36	3.3 to 9.6

boutons disappeared as a result of autolytic processes within the period indicated.

The only variable of any magnitude in the technic applied in the present study is the period of time during which the tissues lay in formalin. Counts from two blocks from the same region of H39, one prepared after 23 hours of immersion in formalin and one after 1 year, are listed in Table VIII. The slight difference in the two counts is not considered significant enough to indicate a diminution in the number of terminals due to the action of formaldehyde. Obviously, this one instance was a poor control for the entire series, but no additional means of checking the point with human material was available.

Of considerable interest in an analysis of this kind is the effect of age on the number of demonstrable terminals. That aging and its attendant wear have some effect on the morphology of boutons in nonneuropathologic cases has been reported.[11] The distribution of the counts in the present series by number of terminals per cell section is indicated in Table IX in two age groups, under and over 30 years. The small number of nonneuropathologic cases in the younger group prohibits any conclusions from these comparisons but certain factors

may be mentioned which may have a bearing on such a disposition. The younger group included four neuropathologic cases and two non-neuropathologic cases under 1.5 years, which age group has previously been described as poor material for demonstrating boutons.[10] In addition, the relatively greater proportion of high counts for neuropathologic cases in the younger group may be related to the nature of the pathologic processes, a large proportion of these being inflammatory (meningitis, myelitis).

TABLE VII

Comparison of Number of Boutons per Cell Section in Cases Subject to Autolytic Influence of More Than 13 and Less Than 7 Hours

	Sensory and internuncial			Nucleus dorsalis			Intermedio-lateral			Somatic efferent		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
More than 13 hours (2 sections)	100	318	3.18	53	283	5.34	29	79	2.72	99	764	7.71
Less than 7 hours (3 sections)	105	272	2.59	71	419	5.90	54	141	2.61	157	1001	6.38

A more adequately controlled comparison by nuclear columns in nonneuropathologic material (from cases in a previous report[11]) is shown in Table X, with three sections counted from cases 9 and 14 (ages 11 and 20) and three sections from cases 25, 27 and 32 (ages 46, 50 and 54). All sections were from the thoracic region, all were subject to less than 8 hours of autolysis and all were submitted to the same technic. No lesions of the central nervous system were evident in any of the cases. The disparity in the counts of endings around the cells of the nucleus dorsalis and the somatic efferent column would suggest

TABLE VIII

Comparison of Number of Boutons per Cell Section Counted in Same Case Subject to Different Periods of Treatment with Formalin

	Dorsal horn			Ventral horn		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
H39 Lumbar Formalin 23 hours	372	1518	4.08	264	1858	7.04
H39 Lumbar Formalin 1 year	231	892	3.86	248	1650	6.65

that the former were increased and the latter diminished with age. Certain points which might clarify such a relationship will be introduced later in the discussion of spatial influence on terminal change.

It is concluded from the group data above that pathologic processes may effect either an increase or a decrease in the number of terminals

TABLE IX

Distribution of Total Counts Arranged in Two Age Groups

No. of boutons per cell section	Under 30 years	30 years and over
0–1	x x x o	x x x x x x
1–2	x x x o	x x x x
2–3	x x x x x o	x x x x x x x x o o o o
3–4	x x x x x o o	x x x x x x x x x x x x o o o
4–5	x x x x x x x	x x x x x x x x x x x o o o o o
5–6	x x x x	x x x x x x x x o
6–7	x x x x	x x x x o
7–8	x	x
8–9	x	x
9–10	x	x
10+	x	x

o = nonneuropathologic; x = neuropathologic.

in the spinal cord. In evaluating such an alteration the age of the individual must be considered.

4. ALTERATIONS IN THE MORPHOLOGY OF CENTRAL NERVE TERMINALS

It seems expedient at this point to insert observations on morphologic variabilities in the boutons in the neuropathologic cases. Preliminary observations of morphology in nonneuropathologic synapses have been reported [11] and it would seem permissible to assume that the conclusions for these cases apply, as well, to the group to be presented. These conclusions are: that variations in bouton "form" may be expected which are unrelated to the pathology, and that comparative data must be controlled as to region examined, nuclear column and age. Certain

fundamental conceptions of the nature of alteration at the synapse are ascertainable only from group data. These are essential to the interpretation of data from individual cases to be described later.

The distribution of the boutons by "type" (Figs. 1, 2 and 3, and schema), expressed as percentages of the total number counted in 26

TABLE X

Comparison of Number of Boutons per Cell Section in Two Age Groups (Nonneuropathologic)

	Sensory and internuncial			Nucleus dorsalis			Intermedio-lateral			Somatic efferent		
	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section	Cell sections	Boutons	Boutons per cell section
Under 20 years (3 sections)	164	382	2.33	188	401	3.40	77	206	2.67	147	920	6.26
Over 45 years (3 sections)	131	322	2.46	64	326	5.09	47	115	2.45	124	611	4.93

nonneuropathologic cases and in 20 cases with damage to the central nervous system are given in Table XI. These data indicate that the fundamental "types" of nerve terminal configurations are not disturbed under pathologic influence.

In Table XII the percentages of regular, thickened and granular "forms" (Figs. 1, 2 and 3, and schema) of boutons in the same two

TABLE XI

Comparison of the Proportionate Distribution of the Five "Types" of Boutons in Nonneuropathologic and Neuropathologic Spinal Cords

	Type 1 Small loops	Type 2 Large loops	Type 3 Filamented loops	Type 4 Fibrillated bulbs	Type 5 Opaque and granular bulbs
Nonneuropathologic (26 cases)	46.36%	31.04%	0.67%	19.68%	2.23%
Neuropathologic (20 cases)	45.49%	31.97%	1.01%	19.51%	1.95%

groups are compared. Approximately 12 per cent more granular forms and correspondingly fewer regular forms occurred in the neuropathologic cases. As in the comparisons reported for the effects of technic, autolysis and aging in nonneuropathologic cases,[11] the thickened forms occurred in constant proportion. Because of this consistency in the appearance of thickened terminals (representing increased silver affinity) these structures may not be considered an indication of pathologic change in the human spinal cord (see, however, Hoff [7, 8] and

Gibson [19] for criteria of alteration in the cat). In the comparisons to follow the thickened terminals will be included as regular forms.

Since age must be considered in relation to the proportion of granular boutons appearing in section, it is important to note the differences in Table XIII in which the cases are subdivided into age groups over

TABLE XII

Percentages of Regular, Thickened and Granular Boutons (Forms) in Nonneuropathologic and Neuropathologic Spinal Cords

	Regular	Thickened	Granular
Nonneuropathologic	79.64%	4.70%	15.66%
Neuropathologic	68.22%	4.27%	27.51%

and under 30 years. As might be expected from the previous report,[11] the difference between nonneuropathologic and neuropathologic cases is more striking in the younger group. The total average percentages for forms of terminals in the entire series of neuropathologic cases (except H113, H114, H115) were 31.41 per cent granular and 68.59 per cent regular boutons of 40,660 counted around 9700 cell sections. These

TABLE XIII

Percentages of Regular and Granular Boutons Appearing in Nonneuropathologic and Neuropathologic Cases in the Age Groups Under and Over 30 Years

	Regular boutons	Granular boutons
Under 30 years:		
Nonneuropathologic (5 cases)	92.01%	7.99%
Neuropathologic (9 cases)	75.15%	25.67%
Over 30 years:		
Nonneuropathologic (21 cases)	78.23%	22.20%
Neuropathologic (11 cases)	71.25%	28.75%

data indicated that, in general, granular forms of terminals appeared in greater proportion in cases with involvement of the central nervous system, and suggested, in view of the similar finding in aging, that the influential processes incident to both aging and direct pathology may be the same.

It should be noted here that the term, *granular,* is inadequate as a description of these altered terminals. Vesiculation, particularly of fibrillated bulbs, and swelling and paling of the neurofibrils are also

conspicuous features (Hoff,[7,8] Gibson,[19] Marinesco,[20] Foerster, Gagel and Sheehan,[21] Schimert,[22] Snider [23]). Similar changes in neurofibrils of central nerve fibers (gn, Fig. 3) have been described in experimental poliomyelitis by Hurst.[24] Different degrees of bouton alteration are illustrated in Figures 1, 2 and 3. In Figure 1 all terminals are regular or thickened with the exception of the granular loop S″. In Figure 2 a relatively greater proportion of granular forms is in evidence and in Figure 3 nearly all the boutons appear granular. Figures 1 and 2 are from case H43, Figure 1 representing boutons around an intermediate sensory and internuncial cell, Figure 2 the terminals around an anterior horn cell. Figure 3 shows the terminals around the axon root on a cell of the nucleus dorsalis in case H7.

TABLE XIV

Group Data Showing Variations in Morphology and Number of Boutons, Arranged Categorically by Pathologic Processes

Myelitis or myelopathy	Cell sections	Total boutons	Per cent granular boutons	Represents increase over control average	Boutons per cell section
Arteriosclerotic (9 cases)	977	4598	21.84	3.31%	4.71
Metabolic, malnutrition, inanition (11 cases)	922	3889	29.83	11.30%	4.22
Toxic (11 cases)	1047	2301	31.46	12.93%	2.20
Neoplastic (10 cases)	1740	7214	31.67	13.14%	4.15
Traumatic (12 cases)	2382	10434	33.18	14.65%	4.38
Meningitis (11 cases)	1286	6410	35.32	16.79%	4.98
Other inflammatory (14 cases)	1469	6612	40.74	22.21%	4.50

5. THE INFLUENCE OF THE FUNDAMENTAL NATURE OF THE PATHOLOGIC PROCESS ON BOTH NUMBER AND MORPHOLOGY OF NERVE TERMINALS

The data of Table XIV indicate that quantitative differences in intensity or degree of alteration in both number and morphology of boutons may be related to the nature of the involvement. The values for the arteriosclerotic group are summarized from cases H77, H81, H55, H23, H18, H12, H6, H3, H31; those for the metabolic, malnutrition and inanition group, from cases H86, H11, H40, H44, H96, H88, H105, H104, H97, H9, H24; for the toxic group, from cases H56, H51, H47, H48, H60, H112, H73, H100, H103, H92, H82; for the neoplastic myelopathies, from cases H29, H37, H45, H17, H8, H49, H42, H50, H64, H62; for the traumatic group, from cases H1, H39, H16, H58, H74, H76, H75, H83, H91, H70, H99, H111; for meningitides,

from cases H7, H53, H20, H27, H87, H102, H71, H98, H93, H85, H84; and for the other myelitides, from cases H5, H43, H41, H72, H109, H107, H89, H108, H110, H106, H94, H90, H101, H95. Inspection of Table I for the essential data of the cases in these groups will reveal a highly arbitrary selection. The placing of the three cases of old poliomyelitis in the group indicating traumatic effects requires the notation that the present lesion was considered a repair effect after loss of cells, the acute pathology having long since gone. Likewise, the diabetic cases in the metabolic group could undoubtedly be placed with the arteriosclerotic cases as well. With such limited data it was almost impossible to find exactly parallel cases adequate for comparison in a controlled series in order to detect effects due to differences in fundamental pathologic processes. Other than the general fact of variability in both number and morphology of terminals in the diseases arranged categorically, little may be said until the individual case data have been described. The striking diminution in the number of boutons per cell section in the toxic group was due to the fact that scarcely any boutons were demonstrable in the two cases of tetanus (H60, H112). The cases are regrouped according to synaptic pattern in section 9.

6. INFLUENCE OF THE DURATION OF THE PATHOLOGIC PROCESS ON THE NUMBER AND MORPHOLOGY OF TERMINALS

A suggestion that duration of the pathologic process may be importantly related to alterations in morphology of boutons is apparent in comparing the values for the acute diseases (particularly those inflammatory) with the values for chronic diseases (arteriosclerotic) in Table XIV. These two groups of cases may be thought of as similar with respect to general, widespread involvement but differ as to age and in the nature of the pathologic influence. The neoplastic and traumatic groups may not be included in such a comparison because of the specific location and extent of the lesions involved. More appropriate comparisons to illustrate the point are those in Table XV. The first compares the counts on anterior horn cells in 3 cases of poliomyelitis (H110, H90, H95), with a duration of 3 days or less, with counts on similar cells in 5 cases of the same disease (H101, H106, H94, H108, H41), with a duration of over 5 days. The second comparison illustrates the effect of duration of pathology on the cells of the nucleus dorsalis in 11 cases of meningitis subdivided into acute (cases H84, H85, H93, H7), subacute (cases H53, H102, H87, H98) and chronic (cases H20, H27, H71). In the subacute group all sections were histologically inflammatory, although the principal affection clinically was purulent meningitis. The values listed for boutons per cell section are averages of similar indi-

vidual notations in each category. It is evident that alteration in both number and morphology varied with the duration of the pathologic process. It is apparent, too, that change in number of boutons did not necessarily correspond in direction with morphologic change. Points that clarify these detailed discrepancies will be introduced in the discussions of individual cases.

7. THE INFLUENCE OF THE DISTANCE BETWEEN THE LESION AND THE ALTERATIONS OBSERVED

Referring to Table X it will be noted that endings adjacent to cells of the nucleus dorsalis appeared to be more numerous and those around somatic efferent groups less numerous in older individuals. Since

TABLE XV

Comparison Within Disease Categories, Illustrating the Influence of the Time Element on Synaptic Relationships

	Duration	Anterior horn cell sections	Boutons	Per cent regular	Per cent granular	Average no. boutons per cell section
Poliomyelitis (8 cases)	Less than 3 da. (3 cases)	155	1185	59.41	40.59	7.80
	More than 5 da. (5 cases)	291	1221	52.98	47.02	4.43
		Nucleus dorsalis cell sections				
Meningitis (11 cases)	Acute, less than 3 wks. (4 cases)	130	996	49.80	50.20	11.40
	Subacute, more than 4 wks. (4 cases)	93	228	52.83	47.17	2.88
	Chronic (3 cases)	45	341	65.85	34.15	7.71

Gardner [25] has shown that a diminution of myelinated dorsal root fibers may be expected after the third decade, the data mentioned above would appear to be erroneous unless it be assumed that the terminals of degenerating axons may persist. The boutons adjacent to the somatic efferent cells are probably, for the most part, terminals of short internuncial cells. The loss of the latter in the presence of persistent terminals of very long fibers suggests that related fiber length may have an important bearing on terminal change. This relationship is further suggested in the comparison of counts in acute and subacute cases of meningitis (Table XV). Where the irritative lesion was extramedul-

lary and confined to the entering fibers (acute cases) the terminals adjacent to the cells of the nucleus dorsalis were altered morphologically but did not seem to be diminished in number (actually increased); on the other hand, in the subacute cases (with intramedullary pathology) there appeared to be a diminution in number of terminals with approximately the same proportion of remaining terminals altered morphologically.

An opportunity to observe terminal effects from loss of cells with relatively short axons was afforded by the three cases of parenchymatous cortical cerebellar atrophy (H113, H114, H115). The pathologic manifestations in this disease include the disappearance of Purkinje cells from the cerebellar cortex with persistence of the basket terminals identifying the positions of degenerated cells (Parker and Kernohan[26]). In Table XVI data from these cases are compared with data from

TABLE XVI

Purkinje Cell Counts in the Cerebellar Cortex Correlated with Terminal Counts in the Dentate Nucleus of the Same Side

	Purkinje layer			Dentate nucleus			
	Normal cell sections	Degenerated cell sections	Per cent cells lost	Cell sections	Boutons	Boutons per cell section	Per cent boutons lost
Control (3 uninvolved)	150	0	0%	100	130	1.30	0%
Parenchymatous cortical cerebellar atrophy (3 cases)	41	261	86%	300	142	0.47	62%

uninvolved cases. It appears, assuming that all terminals ending around cells of the dentate nucleus are from Purkinje cells, that loss of Purkinje cells is reflected in the diminished number of boutons ending in relation to cells of the dentate nucleus. It is possible that all of the terminals of these cells do not disappear with degeneration of the cells (see Cajal,[2] for persistence of axon parts after section). Purkinje cell counts from flocculus and inferior vermis were excluded since the terminals of these cells probably end in relation to cells of the cerebellar roof nuclei.

8. PROGRESSIVE AND RETROGRADE EFFECTS AT THE SYNAPSE

That changes in boutons occur when their associated axons have been damaged experimentally is concluded from the work of Lawrentjew,[3] Gibson,[4] Hoff,[7,8] Foerster, Gagel and Sheehan,[21] Schimert,[22] Snider,[23] Hoff and Hoff[27] and Fulton, Hoff and Kennard.[28] These authors have agreed on the general principle of progressive degenera-

tion detectable at the synapse, but not completely as to the characteristics of the degenerative change. Phalen and Davenport [29] inclined to the belief that the variability in appearance at the synapse precludes the use of morphology as a criterion of degeneration. Barnard [9] failed to find progressive degenerative changes after appropriate experimental manipulation. He found, however, that boutons diminish in number following section of the axons of the cells about which they terminate (retrograde effect). This "axon reaction" is denied in the experimental work of Lawrentjew,[8] Barr [30] and Schadewald.[31]

The change in the terminals around cells of the nucleus dorsalis in the presence of an irritative lesion at the dorsal roots (Table XV, acute meningitis), and the diminution in number of terminals about cells of the dentate nucleus with loss of Purkinje cells (Table XVI) support the conclusion that alteration in boutons at the synapse is related to damage of the parent cell body or axon (progressive). By no means, however, is it implied that this alteration must, in every case, be considered degenerative. On the contrary, the alteration in boutons seems to be related frequently to increased synaptic activity, *e.g.*, increased number of terminals (beading effect) in early poliomyelitis (Table XV).

Data from case 53 in the previous report [11] indicated that axon reaction was absent around cells of the posterolateral group in the lumbar enlargement 5 months after amputation of the leg of the same side. Case H16, amputation of left leg 26 years previously, likewise showed no differences in counts of terminals around cells of appropriate columns of both sides in the lumbar enlargement. Further evidence that axon reaction appears to be absent in the human central nervous system is presented in Figures 4 and 5. The photomicrograph in Figure 4 shows persistent basket terminals at the sites of degenerated Purkinje cells in case H115 whose symptoms of Purkinje cell loss existed for 18 years (Table I). Figure 5 shows boutons adjacent to an anterior horn cell undergoing neuronophagia in a case of poliomyelitis (H106). Certain of these terminals are altered morphologically but it would seem this must be related to inflammatory effect on the cells or fibers supplying the afferent components of the synapse. To check this further, cells have been counted according to their appearance (uninvolved, damaged, or degenerated) at the time of examination in seven cases of poliomyelitis, and the nature of the terminals ending in relation to the cells noted (Table XVII). It is evident from the data that the alterations in the terminals are unrelated to the condition of the receptive component about which they end. Some terminals appeared to be engulfed by the neuronophagic cells, which may account

for the slight diminution in the number of boutons per cell section around degenerated cells. It is concluded that retrograde transsynaptic effect is not demonstrable in human material with the technic used in the present study.

9. INDIVIDUAL CASE DATA

The data in Table XIV indicate that quantitative differences in both the distribution of bouton configurations and the number of terminals per cell section appear when cases are grouped according to common pathogenesis. These data represent the total average of the synaptic effects across an entire section and, in so doing, fall short of an accurate description of the pattern of synaptic change which, in many instances, appears to be peculiar to certain diseases. In the descriptions to follow the diseases will be grouped, in so far as possible, according to common alterations at the synapse. Text-Figures 1 and 2, representing the regional distribution (percentage of granular forms) in nonneuropathologic cases under and over 30 years of age, are control graphs for the comparisons to follow.

TABLE XVII

Comparisons Illustrating Absence of Retrograde Transsynaptic Effect at the Human Synapse

Appearance of cell section	Number of cell sections	Number of boutons	Per cent granular boutons	Boutons per cell section
Uninvolved	384	1466	35.61	3.82
Damaged	216	817	39.41	3.78
Degenerated	216	762	39.37	3.53

(*a*) *Poliomyelitis*

The counts in eight cases of poliomyelitis are listed by region and nuclear column in Table XVIII. The essential data for each case may be obtained from Table I. It will be noted that there is considerable variation from case to case. Certain circumstances suggest that these differences may be related to position of the lesions. For example, there was a relatively large number (49.70 per cent) of altered terminals on the cells of the sensory and internuncial groups in case H106 which was peculiar in having an involvement of the dorsal root ganglia (Table I). The general pattern of morphologic change at the synapse in poliomyelitis is represented by the graph (Text-Fig. 3) of case H108 from Table XVIII. Since the cases in this series are under 30 years of age they may be compared with Text-Figure 1.

It has been mentioned (Table XVII) that the alterations in the

terminals do not appear to be related to the condition of the cells about which they end. With the supposition, then, that all bouton changes are related to influence on the parent cell or axon it is interesting to note the condition of the cells in the various columns and translate this to synaptic effect. Of 449 anterior horn cells counted in 7 cases of poliomyelitis, 310 (69 per cent) were damaged or degenerated. On the basis of the above premise the bouton effect from this injury should be evident at the peripheral end-plates (Carey [5]). Of 367 sensory and internuncial cells counted in the same series, 122 (33 per cent) appeared damaged or degenerated. In this series an average of 44 per cent of the terminals adjacent to anterior horn cells appeared granular (Table XVIII). This represents an increase of approximately 36 per cent in granular forms in this location over the control averages (Text-Figs. 1 and 3). The similarity between the proportion of damaged sensory and internuncial cells (33 per cent) and the proportion of altered terminals adjacent to cells of the anterior horn (36 per cent) suggests that most of the boutons in relation to efferent cells have their source in internuncial columns. An inflammatory process of the nature of poliomyelitis might affect axis cylinders and their ramifications without observable change in the distant parent cell body. Hurst [24] has demonstrated granulation of neurofibrillae of unknown sources in experimental poliomyelitis. In the present material, granularity of neurofibrillae, appearing as a beading effect, was common in the neuropil in inflammatory cases.

The 3 cases (H70, H99, H111) of old poliomyelitis are of interest chiefly in demonstrating the persistence of bouton formations adjacent to glial scars identifying the position of degenerated cells. In cases H70 and H111 in which the condition had existed for 73 and 50 years, respectively, the involved side stained lightly and the cell scars were difficult to identify with certainty. In case H99, however, the scars were prominent (presumably glial replacement) and around 32 of these scars, 58 boutons have been identified.

(*b*) *Inflammatory Myelitides Other Than Poliomyelitis*

This group includes cases which are fundamentally similar to the above although they vary in etiology and in certain histologic details. Included are Landry's paralysis (H5), hemorrhagic encephalomyelitis (after Baker [32]) (H107), postinfectious encephalomyelitis (H43), postmeasles encephalitis (H109), actinomycotic myelitis (H89) and abscess (H72). Histologically, these cases were all inflammatory and many showed the neurofibrillar beading effect described for poliomyelitis. The bouton distributions for these cases are listed in Table XIX.

TABLE XVIII

Bouton Distribution in Poliomyelitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H94	L	47	125	30.40	2.66									55	341	36.07	6.20
H108	T	36	13	30.74	0.36	22	23	39.12	1.05	28	22	31.82	0.78	59	80	68.75	1.36
H41	T	20	55	36.36	2.75	8	20	60.00	2.50	6	25	40.00	4.17	25	146	58.90	5.84
H110	L	60	60	21.66	1.00									70	584	42.46	8.34
H106	S	47	167	49.70	3.55									64	316	37.34	4.94
H90	C	58	70	44.29	1.21									39	252	52.38	6.46
H101	L	58	187	25.67	3.22									88	338	34.02	3.84
H95	L	39	118	22.03	3.03									46	349	26.93	7.59

* L = lumbar; T = thoracic; S = sacral; C = upper cervical.

TABLE XIX

Bouton Distribution in Inflammatory Myelitis Other Than Poliomyelitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H5	T	44	126	25.39	2.86	18	67	28.35	3.72	20	103	43.68	5.15	55	560	62.85	10.18
H43	T	27	66	25.75	2.44	12	52	25.00	4.33	12	29	17.24	2.42	35	526	41.44	15.02
H43	L	17	59	25.42	3.47	8	54	12.96	6.75					22	350	43.14	15.90
H109	L	59	205	55.12	3.47									55	564	37.76	10.25
H89	T	13	17	52.94	1.31	17	30	23.33	1.76	25	18	50.00	0.72	22	120	60.83	5.45
H72	C	23	5	60.00	0.22									33	18	72.22	0.55
H107	L	34	137	48.17	4.02									43	212	18.38	4.93

* T = thoracic; L = lumbar; C = upper cervical.

The variations in the group are extreme, and it will be noted by comparing with Table XVIII that only cases H5 (Landry's paralysis), H43 (postinfectious encephalomyelitis) and H109 (postmeasles encephalitis) exhibited a synapse pattern similar to poliomyelitis. The synapses of case H107 (hemorrhagic encephalomyelitis) appeared to be affected primarily in the dorsal horn. Cases H89 and H72 (bacterial myelitides) showed extreme necrosis in cord sections. It is interesting to note the apparent increase in number of terminals ending in relation to the somatic efferent cells in cases H5, H43 and H109. Of 147 internuncial cells counted in these cases, only 4 were completely degenerated at the time of observation while 81 were demonstrably altered (fragmented neurofibrillae, eccentric nuclei, swelling). In these cases the beading effect in neurofibrillae of the neuropil was prominent, suggesting that the apparent increase in contact points at the ventral horn synapses may be a peripheral manifestation of beading along the parent stem. Case H109 had a concomitant meningeal exudate which may account for the relatively large proportion of granular boutons on the cells of the dorsal horn (see Meningitis). The proportionate distribution of terminal forms appearing in H5 (Landry's paralysis) is illustrated graphically in Text-Figure 4. The total averages for the counts in the cases of inflammatory myelitis are included in Table XIV.

(*c*) *Meningitis*

Certain differences in the synapses within this group have been pointed out (Table XV, second part). Counts of the individual cases are listed in Table XX. The averages for the entire group indicate that the predominant synaptic effect was on the terminals of the dorsal horn (Fig. 3, Plate 165, taken from case H7). The central reactions were obviously less marked in the group with no purulent exudates (H20, H27, H71), and the effect in the dorsal horn appeared to be diminished somewhat in case H84 in which continuous drainage had been instituted. In keeping with the previous statement that terminal change is related to influence on the parent stem, the assumption is that the meningeal exudate is responsible for much of the intramedullary change in the cases of this group. Such influence should be manifest only on the terminals of the dorsal root fibers intramedullarly, since irritation of the ventral roots would not effect an alteration transsynaptically. It has been mentioned that many of the cases of purulent meningitis, particularly those persisting for several weeks, show intramedullary lesions of an inflammatory nature. Excluding cases H7 and H53 and the chronic meningitides, 467 cells have been counted, 231 or 49 per cent of which are pathologically altered. Obviously, the synaptic pattern in these

TABLE XX

Bouton Distribution in Meningitis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H84	T	21	94	34.04	4.47	27	90	32.22	3.33	22	24	16.67	1.09	25	258	44.96	10.32
H84	C	46	279	56.63	6.06									35	491	48.88	14.02
H85	L	19	145	56.55	7.63	6	188	60.63	31.33					23	535	35.51	23.26
H93	T	14	53	56.61	3.78	23	40	60.00	1.74	18	32	28.12	1.77	26	124	30.64	4.77
H7	T	116	354	16.67	3.05	74	678	47.93	9.16	20	56	5.35	2.80	92	716	6.84	7.78
H53	T	38	64	32.81	1.68	28	61	45.90	2.18	8	14	21.43	1.75	52	223	13.90	4.29
H102	T	44	113	48.67	2.56	26	51	49.02	1.96	24	24	25.00	1.00	44	182	42.30	4.14
H87	T	20	25	48.00	1.27	17	67	44.78	3.95	35	41	26.83	1.15	35	138	44.93	3.94
H98	T	25	29	37.92	1.16	22	49	48.97	2.22	18	17	23.52	0.94	28	63	52.38	2.25
H20	T	23	53	24.53	2.30	19	114	20.56	6.00	6	14	7.14	2.33	24	137	13.87	5.70
H27	T	18	50	8.00	2.78	9	72	25.00	8.00	7	23	39.13	3.29	18	138	22.46	7.66
H71	T	34	91	40.66	2.68	17	155	52.91	9.12	12	17	23.53	1.42	28	219	36.07	7.82

* T = thoracic; C = upper cervical; L = lumbar.

cases must be considered that of a combined meningitis and myelitis (compare with case H109, Table XIX). An apparent increase in the number of terminals (beading effect) was evident in certain of these purulent lesions (H84, H85) which had not persisted over 3 weeks (Table XV). Cases H7 and H53 showed little intramedullary alteration. The distribution of granular forms of terminals in case H7 is shown in Text-Figure 5. This was a case of purulent meningitis with a minimum of intramedullary pathology. The pattern in case H102, presenting combined meningitis and myelitis effects, is shown in Text-Figure 6 (compare Text-Fig. 5 with Text-Fig. 1, and Text-Fig. 6 with Text-Fig. 2).

TEXT-FIGURE 4. Granular bouton distribution in a thoracic field of case H5, Landry's paralysis. (See Text-Fig. 1 for further explanation.)

TEXT-FIGURE 5. Synaptic pattern of a thoracic field in case H7, purulent meningitis.

TEXT-FIGURE 6. Synaptic pattern of a thoracic field in case H102, meningeomyelitis.

Case H84 is of particular interest in that the meningeal exudate had been drained from the lumbar region. In the thoracic section counted (Table XX), very little exudate was in evidence and the greater synaptic effect appeared on cells of the somatic efferent group. At this level 95 cells were counted and only 1 was demonstrably pathologic. In the cervical section of the same cord, 81 cells were counted and 58 of these were undergoing pathologic change. This suggests a regional relationship between exudate and underlying intramedullary pathology. Further, the diminished change in the terminals around cells of the nucleus dorsalis relative to alteration around cells of the sensory and internuncial groups (compare with others in the same series) suggests a separate source for the boutons of these two columns. The coincidence of absence of exudate at the lumbar enlargement appears to support the contention that the nucleus dorsalis is, at least in part, a receptive group for fibers entering in that lower region. Alterations at the thoracic anterior horn synapses may be related to the intramedul-

lary damage at higher levels. The absence of marked synaptic change at the somatic efferent cells in case H7 (Text-Fig. 5) is considered to suggest that few, if any, dorsal root fibers pass directly to the ventral horn cells. This supports the contention mentioned above that internuncial cells seem to be the main source of the distributing components to the final-common-path cells. The evidence for this from group data in cases of meningitis is not striking due to the common presence of intramedullary pathology.[8, 9, 27, 28]

(*d*) *Direct Cord Injury* (*Trauma and Tumor*)

This group of cases includes those in which the spinal cord had been traumatized or invaded in such a manner as to establish a definite lesion. The data from the cases are listed in Table XXI together with the position of the lesions and the regions counted. Case H49 was that of a carcinoma of the lung with metastases to the upper thoracic and cervical vertebrae. There appeared to be no encroachment on the cord itself, the damage probably resulting from involvement of radicular and epidural vasculature. There was uniform, diffuse demyelination throughout the cervical cord. The significant lesion in case H64 was tumor erosion of the lower four lumbar vertebrae with involvement of the cauda and conus but avoiding the spinal cord proper. Weigert preparations showed total demyelination of the dorsal columns at the level of the lumbar enlargement. Case H50 was one of multiple myeloma with destruction of vertebrae T7 and T8 and softening of the cord below this level and partial destruction above up to cord level T7.

It would seem from the data that any conception of a definitely localized traumatic or tumor lesion in the human cord is erroneous. In addition to the generalized synaptic effect in the three tumor cases (H49, H64, H50) it is interesting to note the cellular condition in these cases. Thirty-nine of 56 cells counted in case H49 were damaged. Of 101 cells counted in a section through the cervical enlargement of case H64, 54 were demonstrably altered pathologically. The predominant manifestation at this distant focus was marked vacuolization of the nerve cells. In an upper cervical field from case H50, 35 of 104 cells appeared damaged. In addition to widespread cellular response to these focal lesions, both the neuropil of the gray matter and the neuraxes of the white matter showed marked alteration. Prominent among the changes in these locations were swelling of the neuraxes with some fragmentation and clubbing of the fragmented ends. The beading effect, described for neurofibrillae in inflammatory involvement, was absent in these cases. Lumbar and sacral sections from case H50 were completely necrotic, only 38 degenerated cells with 43 boutons being

TABLE XXI

Bouton Distribution in Cords Which Have Been Directly Traumatized or Invaded by Tumor

Case	Position of lesion*	Region examined*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
			Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H49	C vert.	Ce	24	109	41.28	4.54									32	126	46.82	3.94
H64	cauda equina	L	33	10	40.00	0.30									50	34	70.58	0.68
H64	cauda equina	T	27	52	67.31	1.93	20	72	65.28	3.60	17	32	18.75	1.88	28	87	63.22	3.11
H64	cauda equina	Ce	34	138	60.87	4.06									67	463	53.77	6.91
H64	cauda equina	C	35	106	41.48	3.03									50	282	50.34	5.64
H50	T7–Co 1	T12	31	31	74.19	1.00	22	21	80.95	0.95	2	11	72.72	0.92	31	37	78.37	1.19
H50	T7–Co 1	T7									5	5	60.00	1.00	21	167	43.11	7.95
H50	T7–Co 1	Ce	33	70	34.28	2.12									39	386	39.63	9.89
H50	T7–Co 1	C	50	66	45.45	1.32									54	246	50.40	4.55
H1	C6	T4	49	190	43.16	3.88	24	140	42.23	5.83	30	72	58.33	2.40	38	591	63.46	15.5
H76	T9–10	Ce	26	70	55.71	2.69									56	334	36.53	5.96
H75	C	Ce	25	32	56.25	1.28									36	152	50.64	4.22
H83	C5	Ce	16	31	54.84	1.94									26	126	53.17	4.84
H91	C	T	24	104	57.69	4.33	12	79	41.77	6.58	17	49	26.53	2.88	25	281	50.17	11.24

* C = upper cervical; T = thoracic; Co = coccygeal; Ce = cervical enlargement; L = lumbar.

identifiable at these levels. Sections through the lower thoracic regions were necrotic with neurofibrillar granulation so pronounced as to cloud the certain identity of boutons. As compared with data in Table IV a diminished number of boutons appeared only at levels close to the lesion. This lends support to the conclusion previously stated that nerve terminals of long fibers may persist following degeneration of the parent cells.

Of the fracture cases, all but H76 presented similar pictures which are represented by the pattern in a thoracic section from case H1 in Text-Figure 7. H76 differed in that the lesion was below the region counted. The relatively greater proportion of granular forms of terminals around the dorsal horn cells in this case suggests that ascending fibers had a greater tendency to end in this region than descending fibers. A diagram of the pattern of synapses at the level counted in case H76 is shown in Text-Figure 8. The sections from the fracture cases showed swelling and clubbing of neuraxes similar to that described for the tumor cases, but in general, to a lesser extent.

(*e*) *The Cord Synapses with Cerebral Lesions*

The following cases demonstrate synaptic effects in the spinal cord as related to definitive lesions involving the intracranial structures. They differ from the previous group primarily in the distance of the lesion from the region observed. The counts from this group are presented in Table XXII. Essential data from the case histories may be found in Table I. On general inspection of the data in Table XXII it is evident that great variability existed in the group. Unlike the cases of cord tumor (Table XXI) the cerebral tumor cases did not seem to produce generalized alterations at the cord synapses. As a result it may be permissible to assume that these cases represent actual focal lesions, the effects of which at the cord synapses are due to direct involvement of the parent fibers or cells and not to general toxicity or vascular damage. The following discussions are based on this assumption.

Case H74 was one of hydranencephalus in which only 4 per cent (by weight) of forebrain tissue was present.[33] The boutons in this case (age 3 months) were demonstrated with pyridine silver. As a result their morphologic state cannot be compared with the remaining cases in the group. Certain aberrant characteristics were, however, apparent. The terminals were sparse in a dense pericellular net, and were uniformly small. No typical granular boutons were in evidence. Weigert and Marchi preparations were of little help in defining the tract effect in the cord at this age. Hematoxylin and eosin and silver preparations revealed an abundance of fibers in the region of the corticospinal path-

way, the sources of which were obscure. It is interesting to note that terminals, obviously of afferent or internuncial sources, were demonstrable despite the absence of a cerebral cortex, which undoubtedly has much to do with the development of interneuronal connections.

H42 was a postoperative case of right cerebellar tumor (ependymoma). The count from the right field of a thoracic section is given in Table XXII. A purulent meningitis was evident, clouding whatever retrograde effect might be present in the cord sections. The synaptic picture was that of a meningomyelitis (Table XX, and Text-Fig. 6).

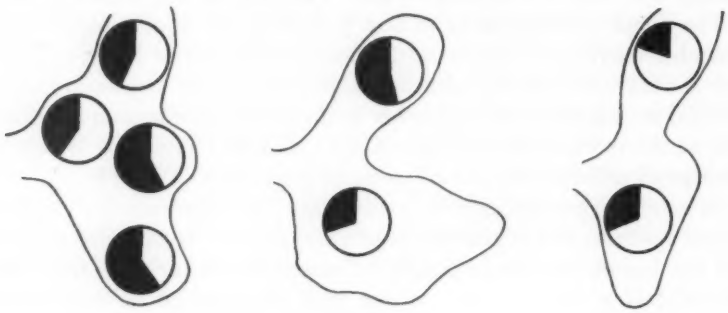
The tumor (astrocytoma) in case H37 filled the third ventricle and extended laterally to obliterate the bodies and anterior horns of both lateral ventricles. It was attached superiorly to the corpus callosum and did not appear to involve other brain tissue. The bouton distribution at the thoracic level (Table XXII) compares favorably with the average of uninvolved cases of the same age group (Text-Fig. 1).

In case H29 an astrocytoma involved the left temporoparietal region at the posterior end of the lateral cerebral fissure. It extended to, but did not include, the basal ganglia. Comparison of the data from the left field of a thoracic cord section with the diagram of uninvolved controls in the same age group (Text-Fig. 2) indicates that no synaptic effect is evident at the level counted.

The tumor (glioblastoma multiforme) in case H45 was described as extending from the central sulcus to within 4 cm. of the right frontal pole and as involving only the anterior limb of the internal capsule. It would appear from the data in Table XXII that the efferent fibers from this area which gained the upper cervical cord (opposite side was counted) ended primarily in relation to cells of the anterior horn at this level. From the description of the tumor site it would seem to involve some of the motor cortex (but not the components which should be delivered to the upper cervical region) as well as most of the premotor cortex and additional frontal lobe. If this be true, it would seem that the effect demonstrated is related to the premotor involvement.[28] The cervical synapses are diagramed in Text-Figure 9.

In case H8 the tumor (glioblastoma multiforme) was described as involving the left superior temporal gyrus and the parietal operculum. From the site of the tumor it would appear to have involved the efferents from the lower part of the motor cortex. A count of the terminals on the cells of the opposite side in the cervical enlargement is given in Table XXII. The data indicate that terminals from this source ended primarily on cells of the sensory and internuncial columns. A sector diagram of the bouton distribution in the opposite field of a section through the cervical enlargement is shown in Text-Figure 10.

Case H17 had a hemorrhagic tumor (extragenital chorioepithelioma) involving the right temporal lobe with softening extending through the right basal ganglia and an additional tumor nodule in the left occipital pole. The right middle cerebral artery was thrombosed. A count of the boutons in a thoracic field of the left side is given in Table XXII. From the description of the lesion it is uncertain whether either limb of the internal capsule was involved. It is unlikely that striofugal fibers descended unbroken to so distant a point.



TEXT-FIGURE 7. Appearance of synapses in a thoracic field in case H1, transverse cervical traumatic myelitis. (See Text-Fig. 1 for further explanation.)

TEXT-FIGURE 8. Synaptic pattern in a field of the cervical enlargement in case H76, transverse thoracic traumatic myelitis. The posterior sector represents terminals on sensory and internuncial cells; the anterior sector, somatic efferent.

TEXT-FIGURE 9. Synaptic pattern in a left upper cervical field in case H45, right cerebral glioma involving premotor cortex and frontal lobe.

The tumor in H62 was an extensive right cerebral astrocytoma involving basal ganglia, both limbs of the internal capsule, and thalamus. The count from a left thoracic field is given in Table XXII and is represented diagrammatically in Text-Figure 11. Unlike cases H45 and H8 in which the lesions were apparently limited to one limb of the internal capsule, the effect on thoracic spinal boutons in this case appeared to be on cells of both the internuncial and somatic efferent groups. On the basis of the evidence from cases H45 and H8 it is possible that the alteration in the terminals around the internuncial groups in case H62 might be related to motor involvement and the changes in the boutons on the efferent cells related to a lesion of the premotor efferents.

Case H12 had a hemorrhage of the posterior right thalamus which extended into the right lateral ventricle. The brain vessels were sclerotic and there were evidences of previous hemorrhages. In view of the age of the patient (75 years) nothing may be concluded from

the spinal bouton distribution (cf. Text-Fig. 2). Although there was a definite cerebral lesion in this case, it might more properly be included in the group with general cerebral arteriosclerosis to be described below.

(*f*) *Toxic Myelitis*

This group includes cases in which involvement of the central nervous system is commonly considered to be due to toxic effects (Table XXIII). Case H82 was diagnosed clinically as encephalitis, but at autopsy as subacute bacterial endocarditis. The effect in case H58 (cystic hypothalamus) was undoubtedly thermal since the temperature rose to 110° F. following appendectomy. In view of the age of the latter patient (18 years), it seems evident that there was a slight bouton effect in hyperpyrexia. The same may be said of case H47 (uremia) in which there was generalized sepsis. The other case (H51), dying in uremia, from seminoma of the testicle with marked infiltration of both kidneys, compares favorably with the uninvolved control (Text-Fig. 1). It would seem, then, that the effect in case H47 was due more to sepsis than to uremia. Case H48 (age 47 years) showed a remarkably uninvolved synaptic picture for the age group (cf. Text-Fig. 2). It is interesting to compare the synaptic pattern of case H82 with those in Tables XVIII and XIX (encephalitis), especially since this case was diagnosed, clinically, as encephalitis. This suggests a relationship between the synaptic picture and the symptomatology. The intramedullary pathology in case H82 included satellitosis, some neuronophagia and slight beading of the neurofibrillae. Considering the age of the patient (20 years), this is a markedly altered picture of the synapses. Case H56 showed nothing unusual for the age group. The two cases of tetanus (H60, H112) were characterized by the reduction in the number of demonstrable boutons per cell section. Neither cord took the silver impregnation well, and the identity of terminals was obscured by the pronounced swelling of the nerve cells, and the granular appearance of the neuropil.

(*g*) *Inanition, Malnutrition, Metabolic Disturbances*

The data from these cases are listed in Table XXIV. With therapeutic measures available for administering food parenterally, it is difficult to obtain a case of frank inanition. Most representative of such a condition in the present series were cases H9 and H24, both with obstructive gastrointestinal lesions and both badly wasted at the time of death. Nothing unusual for the age group was in evidence at the cord synapses, however. Cases H11 and H44 were diabetic patients, the former dying from a cardiac lesion, the latter from pulmonary embolism

TABLE XXII

Bouton Distribution in Spinal Cords With Associated Cerebral Lesions

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H74	T	39	11		0.28	8	11		1.36	41	8		0.19	39	47		1.2
H42	T	37	48	14.58	1.3	27	54	29.63	2.00	9	8	25.00	0.89	27	181	21.00	6.7
H37	T	74	135	7.41	1.82	55	238	3.94	4.33	46	162	4.32	3.52	67	422	8.77	6.3
H29	T	67	267	29.96	3.98	41	232	22.22	5.71	19	102	15.69	5.36	60	330	26.67	5.5
H45	C	38	54	18.52	1.42									44	192	31.25	4.36
H8	Ce	63	169	24.26	2.68									60	425	17.18	7.08
H17	T	35	96	13.54	2.74	35	211	21.80	6.03	20	40	15.00	2.00	40	385	37.14	9.62
H62	T	20	111	61.26	5.55	19	60	28.33	3.15	11	23	17.39	2.09	15	165	47.87	11.00
H12	T	35	88	29.54	2.51	15	105	21.90	7.00	16	41	31.71	2.56	59	341	31.09	5.78
H3	T	35	99	18.18	2.83	40	240	22.08	6.00	25	65	23.08	2.60	62	463	23.33	7.46

* T = thoracic; C = upper cervical; Ce = cervical enlargement.

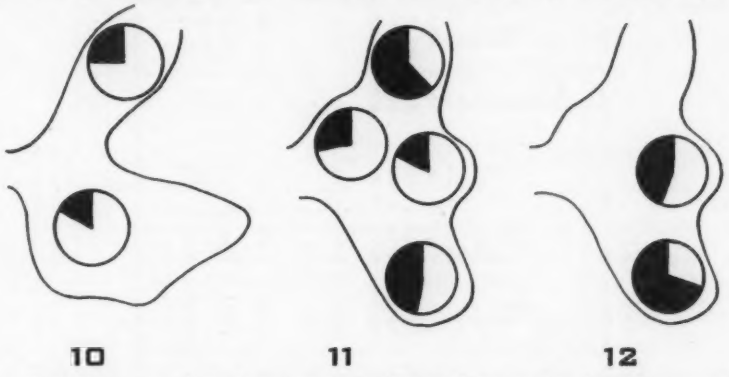
TABLE XXIII

Bouton Distribution at Cord Synapses Subject to Toxic Influence

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermedio-lateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H58	L	36	80	25.00	2.22	11	22	18.18	2.00	20	56	17.85	2.80	43	186	15.05	4.32
H48	T	39	79	8.87	2.02	41	106	13.21	2.58	22	35	11.43	1.59	31	131	20.33	4.22
H47	Ce	29	44	25.00	1.52	9	16	18.75	1.78					73	174	11.49	2.38
H51	S	75	101	7.92	1.35					8	23	8.69	2.87	59	281	5.33	4.76
H82	Ce	34	88	60.22	2.59									42	299	62.88	7.12
H56	T	30	124	29.83	4.13	22	117	29.91	5.32	12	44	20.45	3.67	48	200	20.50	4.17
H60	Ce	33	5	80.00	0.15									73	24	87.50	0.32
H112	L	44	6	100.00	0.13									66	17	88.23	0.25

* L = lumbar; T = thoracic; Ce = cervical enlargement; S = sacral.

following laparotomy. Again, nothing unusual was in evidence at the spinal synapses. According to synaptic patterns, these last two might well be listed with the arteriosclerotic cases below and the first two with uninvolved cases in the older age group. Case H40 (insulin hypoglycemia) showed extensive alteration at the synapses. The lumbar section of this cord presented a picture similar to that described for hypoglycemia by Moersch and Kernohan.[34] Of 62 cells counted, 52 were pathologically altered and 8 completely degenerated. The general diffuse synaptic changes in the cases of malnutrition and deficiency suggest a general pathologic influence, similar in its synaptic manifestations to the myelitides (cf. Tables XVIII and XIX). This group in-



TEXT-FIGURE 10. Synaptic pattern in the right field of a section through the cervical enlargement in case H8, cerebral glioma involving left temporoparietal region. (See Text-Fig. 8 for further explanation.)

TEXT-FIGURE 11. Synaptic pattern in a left thoracic field in case H62, extensive right cerebral tumor. (See Text-Fig. 1 for further explanation.)

TEXT-FIGURE 12. Synaptic pattern in a thoracic field in case H103, necrosis of cord with obliteration of the dorsal horn at this level.

cludes cases H97, malnutrition; H96, vitamin D deficiency; H105, pellagra; H88, avitaminosis A; and H104, pigment degeneration. Of 360 cells counted in the group, 131 were altered pathologically and 5 were completely degenerated, showing a marked cellular, as well as synaptic, effect. Case H73 (questionably diagnosed as pernicious anemia, multiple sclerosis, or old toxic myelitis) showed almost completely degenerated gray matter with only a few shrunken nerve cells, about which were very few boutons. There was diffuse demyelination in the white matter, most prominent in the dorsal columns. The boutons were too few to warrant comment. Cases H100, H103 and H92 represented the disease described by Moersch and Kernohan [35] and Schunk and Kernohan [36] as acute or subacute progressive necrosis of the spinal cord, etiology unknown. In case H103 the dorsal horn was completely

TABLE XXIV

Cord Bouton Distribution in Inanition, Malnutrition, Metabolic Disturbances, and Idiopathic Necrosis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermediolateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H9	T	27	70	12.86	2.59	23	137	21.1	5.95	9	27	18.00	3.00	24	142	9.15	5.92
H24	T	41	99	20.20	2.41	9	50	26.00	5.55	24	53	30.19	2.21	23	161	17.39	7.00
H11	T	36	81	22.22	2.25	34	163	22.09	4.79	29	50	22.00	1.72	47	278	22.66	5.91
H44	T	29	63	23.81	2.17	40	206	23.70	5.15	27	56	21.43	2.07	39	177	23.17	4.54
H40	L	23	84	69.95	3.65									39	238	59.24	6.10
H97	T	18	18	16.66	1.00	10	41	36.57	4.10	19	15	20.00	0.79	36	216	35.64	6.00
H96	L	33	123	38.20	3.73									31	397	25.19	12.80
H105	L	31	50	24.00	1.61									51	200	25.00	3.92
H88	L	23	18	44.43	0.78									29	121	34.20	4.17
H104	T	24	23	21.73	0.96	16	27	25.92	1.68	15	20	25.00	1.34	24	202	34.16	8.41
H73	Ce	25	10	90.00	0.41									40	36	55.55	0.90
H100	T	31	32	37.50	1.03	13	26	50.00	2.00	9	10	30.00	1.11	28	79	53.16	2.82
H103	T									25	16	43.75	0.84	26	97	72.16	3.73
H92	T	14	8	37.50	0.57	12	9	22.22	0.75	6	9	44.45	1.50	28	55	40.00	1.96
H86	C	19	83	42.16	4.37									20	230	45.21	11.50

* T = thoracic; L = lumbar; Ce = cervical enlargement; C = upper cervical.

TABLE XXV
Bouton Distribution in Arteriosclerosis

Case	Region*	Sensory and internuncial				Nucleus dorsalis				Intermediolateral				Somatic efferent			
		Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section	Cell sections	Boutons	Per cent granular	Number per cell section
H3	T	35	99	18.18	2.83	40	240	22.08	6.00	25	65	23.08	2.60	62	463	23.33	7.46
H6	L	75	238	13.87	3.17	30	288	12.72	7.60	14	29	6.89	2.07	62	574	15.67	9.26
H77	Ce	29	5	20.00	0.17									48	9	33.33	0.19
H81	L	21	116	28.96	5.52									14	311	36.01	22.21
H55	S	35	48	27.08	1.37									37	129	39.53	3.49
H33	T	35	85	14.12	2.43	16	74	17.57	4.63	13	35	20.00	2.69	36	197	18.78	5.47
H18	T	25	80	10.00	3.20	23	55	30.91	2.39	15	50	22.00	3.33	37	190	24.21	5.14
H31	T	35	112	25.89	3.20	25	157	16.56	6.28	15	47	14.89	3.13	50	385	16.36	7.70

* T = thoracic; L = lumbar; Ce = cervical enlargement; S = sacral.

necrotic at the level counted, presenting a condition similar to that in the thoracic section of case H50, Table XXI. These two sections were the only ones in the series which seemed to be specifically correlated with alteration of synapses in the intermediolateral column. H103 is represented diagrammatically in Text-Figure 12. H86 was a case of fracture followed by fat embolism. There was diffuse synaptic change.

(*h*) *Arteriosclerosis*

Clinical signs of involvement of the central nervous system were described in the histories of all cases in this group. None, however, showed any definite lesions at autopsy. The bouton counts for eight cases are listed in Table XXV. In general, the synaptic change was uniform and diffuse, although unexplained variations were evident among the boutons of the nuclear columns in some cases. Referring to Table XIV, it would seem these cases were not significantly different from the older age group of uninvolved cases.

SUMMARY

The relationships between distributing and receptive components at the central synapse may be altered demonstrably in the following ways: (1)

damage to, or destruction of, the efferent component (cell body and its processes), which undoubtedly represents a total cancellation of synaptic function, in many cases probably reversible; (2) diminution in the number of terminals representing the distributing component (while this may affect synaptic transfer, there is no evidence that such a reduction operates to cancel it completely); (3) increase in the number of contact points at the synapse which is characteristic of inflammatory involvements and may reflect synaptic hyperfunction; (4) change in morphology of the afferent structures. The significance of this is obscure, but the following possibilities are suggested: (a) the altered boutons may represent a functional state; (b) they may represent degenerated terminals; (c) they may represent persistent terminals of degenerated axons.

Of the factors evaluated for influence in bringing about these changes at the synapse, age and the presence of lesions are the most effective; technic and autolysis do not seem to be importantly related. The intensity of the alterations at the synapse depends upon the fundamental nature of the pathologic process, its duration and on the size of the cells (length of axons, particularly) involved. The manifestations of pathologic influence at the synapse vary with the nuclear group under observation, and certain alterative patterns appear to be peculiar to specific disease groups and to the location of definitive lesions. The observable bouton effects are related primarily to involvement of the parent structure. No retrograde, transsynaptic effect from damage to the efferent component is evident at the human central synapse.

NOTE: Acknowledgment is due Dr. J. W. Kernohan, Division of Neuropathology, Mayo Clinic, through whose courtesy thirty-three of the cases in the present study were made available. Dona Minckler has performed the technical procedures and has assisted with the computations.

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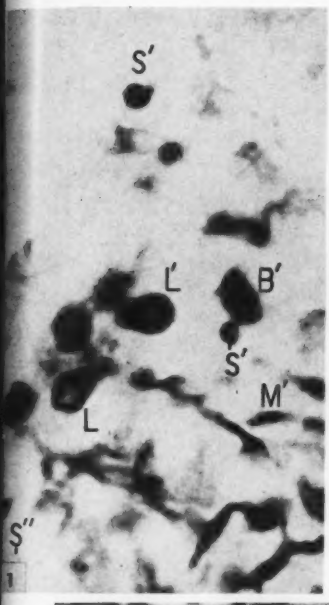
DESCRIPTION OF PLATES

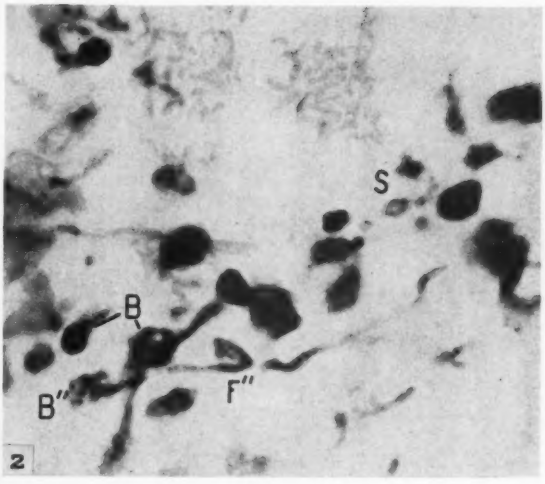
PLATE 165

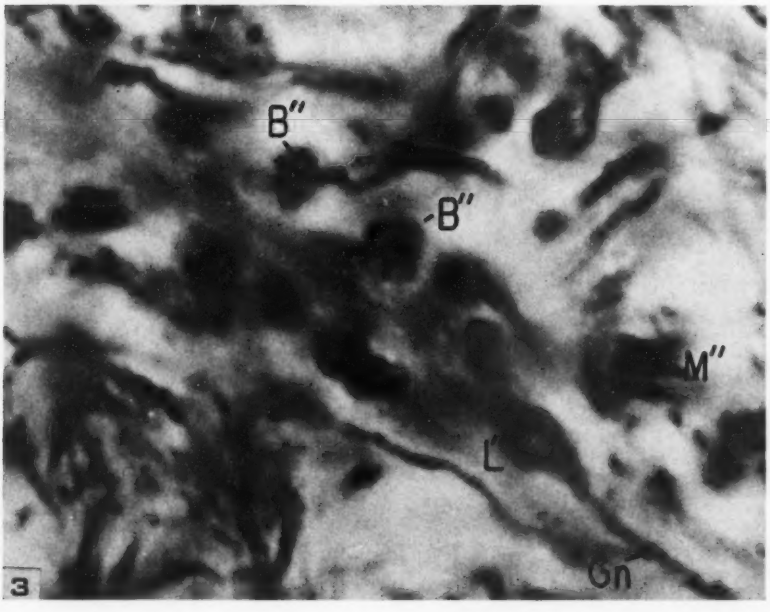
FIGS. 1–3. Photomicrographs of illustrative groups of boutons to which the schema of the morphologic classification as "types" and "forms" refers. Small loops are designated S; large loops, L; filamented loops, F; fibrillated bulbs, B, and opaque and granular masses, M. Thickened forms are marked prime (′) and granular forms, double-prime (″). Gn (Fig. 3) indicates a granular neurofibril. There are different degrees of granulation effect in the three figures. Figures 1 and 2, × 1400; Figure 3, × 1800.

Distribution of Forms

	Regular	Thickened	Granular
Type 1. Small loops	Fig. 2 S	Fig. 1 S′	Fig. 1 S″
Type 2. Large loops	Fig. 1 L	Fig. 1 L′	Fig. 3 L″
Type 3. Filamented loops	none shown	none shown	Fig. 2 F″
Type 4. Fibrillated bulbs	Fig. 2 B	Fig. 1 B′	Figs. 2, 3 B″
Type 5. Opaque and granular masses		Fig. 1 M′	Fig. 3 M″





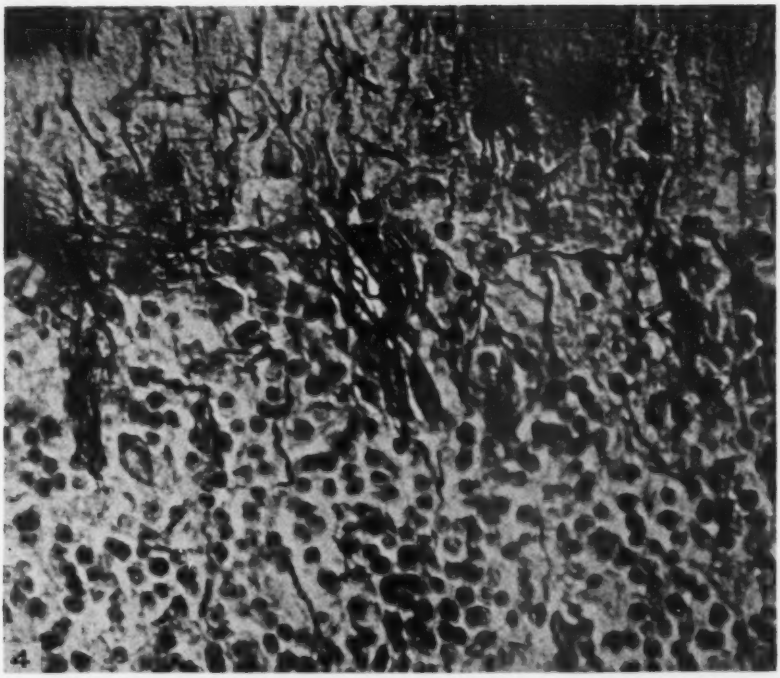


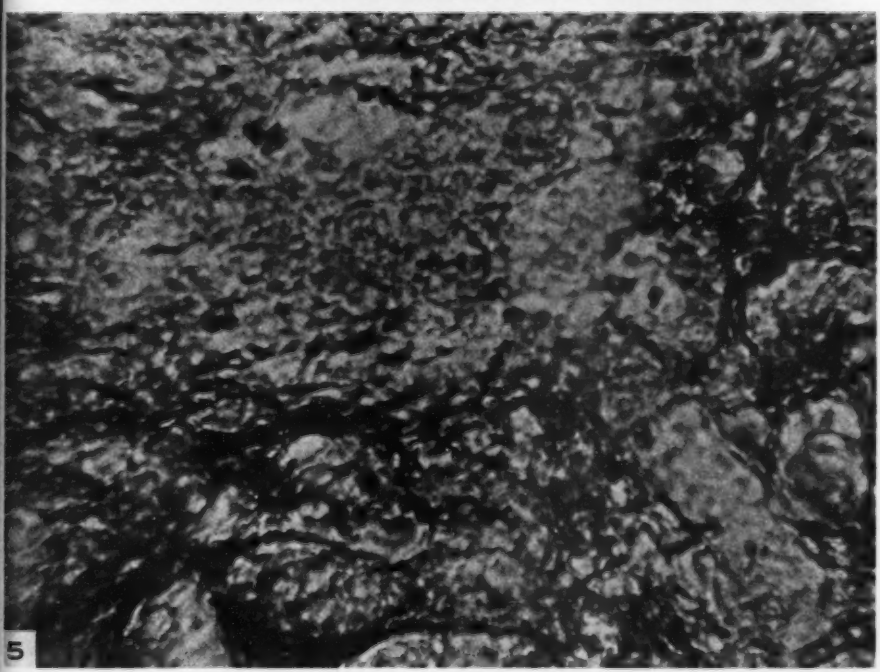
Minckler Pathologic Alterations at the Human Synapse

PLATE 166

FIG. 4. Photomicrograph showing persistent basket terminals following degeneration of Purkinje cells. × 600.

FIG. 5. Photomicrograph showing persistent boutons on an anterior horn cell undergoing degeneration by neuronophagia. × 1200.





Minckler Pathologic Alterations at the Human Synapse

HYPERPLASIA OF MEGAKARYOCYTES IN PNEUMONIA AND ITS RELATIONSHIP TO LEUKOBLASTIC HYPERPLASIA OF THE BONE MARROW *

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In 1908 Dickson [1] stated that the megakaryocytes are, on the whole, somewhat increased in number and size in pneumonia and septicemia, and are less frequently unaltered or diminished. These observations were based on the inspection of the upper part of the shaft of the femur and of the ribs. Frey [2] counted the bone marrow giant cells at the junction of the lower and middle thirds of the femur in 50 cases of widely varying diseases, 10 of which were acute infections. The highest count occurred in a case of polycythemia vera and the next highest in one of pneumonia. He stated that the increase of megakaryocytes in pneumonia is a typical example of the changes of these cells in acute infectious diseases. The findings in his cases of acute infections are difficult to interpret because of lack of suitable controls and because of the bone examined. Nickerson and Sunderland [3] counted the number of megakaryocytes per unit area of vertebral marrow in 3 cases of idiopathic thrombocytopenic purpura, 5 of secondary thrombocytopenic and symptomatic purpura, 2 of unclassified purpura and 8 of sudden death due to varying causes. In this series of 18 cases the highest megakaryocytic count was found in 1 case of idiopathic thrombocytopenic purpura and the next highest count was in one of the cases of secondary thrombocytopenic and symptomatic purpura with pneumonia. Rosenthal [4] stated that in infections, especially pneumonia, there is a marked stimulation and increased formation of megakaryocytes, occurring simultaneously with stimulation and increase of leukopoietic tissue.

Except where stated the above reports were unaccompanied by factual evidence.

MATERIALS AND METHODS

The material was derived from 306 human adults, unselected except for the exclusion of primary hemopoietic diseases. In 39 of these 306 cases, pneumonia of some type had previously been listed in the anatomical diagnosis as the primary disease. This independent consideration of these cases as primary pneumonia permitted an unbiased selection. Only cases of well developed pneumonia were studied, those of terminal bronchopneumonia not being a part of this series. For

* Received for publication, February 6, 1942.

controls a series of 16 cases, those of patients dying of traumatic injuries within 12 hours after injury, was used. Also the clinical records of all cases were surveyed for pertinent data.

Bone marrow from a lumbar vertebra and the upper third of the diaphysis of the humerus was available for study in the 306 cases. In addition to the usual bony sections from the vertebra, vertebral pulp was studied. The latter was obtained by sawing away the cortex of the vertebra and driving a chisel into the spongy bone. The marrow pulp which exuded around the chisel was placed on a small square of filter paper and was then carried through the various technical processes similar to curettings in general.[1] Thus the cytology of nondecalcified vertebral marrow could be studied.

All marrow was fixed in Zenker's-formaldehyde solution, embedded in paraffin and stained with hematoxylin and eosin and with azure II and eosin.[5] Thin slices of spongy bone of the vertebra, cut with a jeweler's saw, were decalcified not over 48 hours in equal parts of an 85 per cent aqueous solution of formic acid and a 20 per cent solution of sodium citrate.[5]

For the purpose of making differential counts of megakaryocytes, sections cut from the paraffin-embedded marrow of the humerus and vertebral pulp were handled according to Wright's technic.[6] In order to control this modified technic, blocks of fresh femoral marrow from rabbits were treated strictly according to Wright's method except that the fixing fluid as altered by Downey[7] was used. Blocks of rabbit's marrow were also treated similarly to the human marrow. By comparing the sections of rabbit's marrow obtained by the two methods with the sections of human marrow, it could be determined whether the latter were technically satisfactory. Figure 1 shows an adult megakaryocyte of the rabbit stained strictly according to Wright's technic after Downey's fixation, and Figure 2 shows an adult human megakaryocyte treated according to the modified Wright's technic herein described.

The terminology in general is that used by Sabin and Miller.[8] Certain explanations are necessary for its adaptation to the material in this study and to the sections stained with azure II and eosin. The primitive cell of this classification is a small round cell scattered throughout the marrow. It differs from the small lymphocyte in having a lower content of chromatin in the nucleus and in lacking rod-shaped mitochondria in the cytoplasm. With the technic and material used here, I was unable, on the basis of cytology alone, to differentiate this cell from the small lymphocyte. Therefore I have called all such cells the small round cells of the bone marrow. The classification of the myelo-

cytes into A, B and C forms is an extremely useful one. Myelocyte C has an oval to slightly indented nucleus. The cytoplasm shows no basophilia and is packed with specific granules. Myelocyte A has less than ten specific granules in its cytoplasm, which is a gray-blue in color. Also included in the myelocyte A group is a cell which represents a transition stage between myeloblasts and myelocytes with only a few specific granules. This cell has a gray-blue cytoplasm which lacks granules. It differs from the myeloblast in having a more abundant cytoplasm and in lacking the intense basophilia of the cytoplasm. This type of myelocyte A is pictured as cell number 15 in Figure 67 on page 82 in Maximow and Bloom's [9] Textbook of Histology. Myelocyte B represents a stage of maturation intermediate between A and C.

Selection of the sites for study of the marrow depends on knowledge of the normal and pathological distribution of the red and fatty marrow in the different bones. According to Neumann [10] (1882), in adults active red marrow is found almost without exception in the skull, vertebrae, sternum, ribs and other bones of the trunk. He stated that in healthy adult persons either all the small and large bones of the extremities contain exclusively fatty marrow or the active red marrow is limited to the upper part of the humerus and femur, that is, the upper epiphysis and a smaller or larger segment of the adjacent diaphysis. There are various transition stages between these two pictures. Piney [11] agreed with Neumann as to the normal state of the marrow of the vertebrae, sternum and ribs in the adult except that he added that by the age of 25 years the marrow of the ribs adjacent to the cartilages for a distance of about 1 inch is fatty. He also agreed essentially with Neumann's findings with respect to the distribution of the red and fatty marrow in the shafts of the long bones in normal adults, stating that in extremely advanced age the diaphyseal patch of red marrow at the upper end of the humerus and femur may be almost completely replaced by fatty marrow. Piney further reported that the shaft of the humerus in adults appears to contain a larger quantity of active marrow than that of the femur. Schmorl [12] emphasized the constancy of hemopoiesis in vertebral marrow as compared to that of all other bones.

Neumann [10] stated that the pathological transformation of fatty marrow into red marrow in the long bones takes place in a centrifugal direction with respect to the trunk. It successively extends not only over the whole humerus and femur but also to the upper epiphysis, diaphysis and lower epiphysis of the bones of the forearm, tibia and fibula, and later to the bones of the hands and feet. He added that the red marrow in the upper extremities appears to extend farther

downward than in the lower extremities. With respect to the diaphysis of the humerus, femur and tibia, I interpret these statements of Neumann in the following manner. The extension of red marrow in the humerus is greater than in the femur and hyperplasia in the femur definitely precedes that in the tibia. Also the hyperplasia begins in the upper end of each bone.

Seecof [13] advanced the concept that hyperplasia does not take place uniformly in the long bones. It occurs first in the humerus, then in the femur and last in the tibia. Later I [14] found, in the examination of the marrow at the junction of the lower and middle thirds of the humerus, femur and tibia in 100 unselected cases, that the presence or absence of hyperplasia, as judged by the sample taken, occurred in certain orderly combinations. Hyperplasia may be (1) absent in all three bones, (2) present in the humerus alone, (3) present in humerus and femur and absent in tibia, or (4) present in all three bones. These data adequately support Seecof's concept. Strumia [15] and Custer and Ahlfeldt [16] noted the tibial marrow to be less active than that of the femur. Seecof,[17] in certain cases of pernicious anemia studied before 1924, found the marrow of the long bones aplastic and the flat bones hyperplastic. This emphasizes the fallacy of examining only the marrow of the long bones. Custer and Ahlfeldt recommended inclusion of the vertebral marrow among the sites to be studied. According to Wienbeck [18] it is necessary to examine the vertebrae in order to interpret the state of the bone marrow.

As the data which follow in this report will show, changes are best detected by a study of the vertebral marrow as compared to that of the upper end of the femur or humerus. This is true because in any individual case the structure of the marrow previous to the onset of disease is best judged in the vertebrae. Therefore, pathological changes can be best detected here. Also cases with hyperplasia in the vertebrae and none in the long bones would be missed by examination of the latter only. The study of the marrow of the vertebrae has certain obvious technical advantages over that of the other flat bones at autopsy.

The above review shows that the examination of the marrow of the humerus has the advantage over that of other long bones of revealing the earliest evidence of hyperplasia in the long bones. Examination of the upper end should reveal the earliest change in the marrow of a particular tubular bone.

For an extensive survey, Custer and Ahlfeldt [16] recommended the study of the marrow of the vertebrae, sternum, rib, femur and tibia. For the same purpose I have studied the marrow of the lumbar vertebrae, the body of the sternum, the upper third of the shaft of the

humerus and the junction of the lower and middle thirds of the femur and tibia. Since the distribution of red marrow differs in an orderly manner, not only in different bones but also in the same bone, care should be taken to examine the marrow at definite locations and proper controls should be available for these particular locations.

Preliminary Findings

By inspection of sections of vertebral marrow from the 306 unselected cases and comparison with controls without any other knowledge of the cases, the presence or absence of an increase of megakaryocytes was determined. Twenty-six cases showed hyperplasia of these cells. The increase was variable throughout the different parts of the same section with a tendency to be marked in focal areas of the marrow, and varied in degree from case to case (Figs. 3 and 4). The vast majority of the megakaryocytes were adult and degenerate with complicated and polymorphous nuclei. Young megakaryocytes were rare. Bizarre mitotic figures [9,19] (Fig. 5) of the megakaryocytes were found in 12 cases of pneumonia and were not searched for in the cases without pneumonia. Seven of these 12 showed hyperplasia of megakaryocytes and 5 did not. No spindles were seen. There was never any evidence of cytoplasmic division associated with these mitotic figures.

Of the 26 cases showing an increase in megakaryocytes, 17, or 65 per cent, were cases of primary pneumonia. Of the 306 unselected cases, only 39, or 12 per cent, were cases of primary pneumonia. This is a significant difference and demonstrates a definite relationship between pneumonia and hyperplasia of megakaryocytes. The 9 cases of hyperplasia of megakaryocytes without primary pneumonia, together with pertinent data, are listed in Table I.

The Control Series

The number of megakaryocytes in the bone marrow was based on the enumeration of these cells in the bony sections of vertebral marrow. The number was much more constant here than in the marrow of the humerus. For example, the marrow of the humerus of case 12 of the controls (Table II) was completely fatty (Fig. 6) and showed no megakaryocytes. That from the humerus of case 1 (Table II) showed the maximum degree of hemopoietic cellularity of the controls for this location (Fig. 7). A considerable number of megakaryocytes were present. Obviously what is normal for the humeral marrow of case 1 would be grossly abnormal for that of case 12. There were all variations between these two examples. The difficulty in detecting hyperplasia of the megakaryocytes in the humeral marrow is apparent because of such wide variations in the normal.

The megakaryocytes were counted per unit area of marrow [20] of the vertebrae and 100 microscopical fields [2] were chosen as the unit in which to count them. Trabeculae of bone were excluded from this area and the more cellular regions were examined. The optical system

TABLE I

Data on Nine Cases of Hyperplasia of Megakaryocytes Without Pneumonia

Number of case	Age	Sex	Diagnosis	Leukoblastic hyperplasia
1	40	M	Recent cholecystectomy for chronic cholecystitis and cholelithiasis; chronic suppurative peritonitis, localized; pulmonary embolus	Present
2	77	M	Diabetes mellitus; carbuncle of neck; pyemia; bronchopneumonia; cardiac hypertrophy and dilatation	Present
3	52	M	Subacute glomerulonephritis; anasarca; hyperplastic lymphadenitis; chronic splenitis; splenomegaly (540 gm.); cerebral hemorrhage; luetic aortitis and valvulitis; chronic passive hyperemia of liver; pulmonary edema; (unclassified hyperplasia of bone marrow; anemia; red blood cells 2.5 million)	Absent
4	44	F	Congenital polycystic kidneys; uremia; chronic suppurative pyelonephritis	Present
5	30	M	Amyloidosis of kidneys; uremia; fibrinous pericarditis; chronic endocarditis; mitral stenosis and insufficiency; cardiac hypertrophy, right ventricle; vegetative endocarditis of mitral valve	Present
6	70	F	Diabetes mellitus; chronic suppurative cystitis; acute suppurative pelvic peritonitis	Present
7	42	F	Recent panhysterectomy and appendectomy for chronic salpingo-oophoritis; generalized peritonitis	Present
8	63	M	Sclerosis of coronary arteries; myocardial infarction, recent; fibrinous pericarditis; cardiac hypertrophy	Present
9	75	M	Carcinoma of the pancreas; sclerosis of coronary arteries; healed myocardial infarction with mural thrombus; cardiac hypertrophy; hyperplasia of prostate gland; dilatation of ureters and pelves; renal calculus; diabetes mellitus; gangrene of right toe; cholelithiasis; (postmortem blood culture, *Streptococcus hemolyticus*)	Present

used was a Zeiss objective 40 with a numerical aperture of 0.65 and 10x eyepiece. From the number of megakaryocytes per 100 microscopical fields the number per cubic millimeter of vertebral marrow was calculated.*

* Since the volume of a cylinder equals $\Pi r^2 h$, if r is the radius of the microscopic field, measured with a stage micrometer, and h the thickness of the section, which was approximately 5 μ, the volume of 100 such cylinders equals $0.165^2 \times 0.005\Pi \times 100$ or 0.043 cmm. Then the megakaryocytes per cubic millimeter of marrow equal:

$$\frac{\text{number of megakaryocytes in 100 fields}}{0.043}.$$

These calculated results are admittedly an arbitrary and not an absolute value of the number of megakaryocytes per cubic millimeter of marrow. Nevertheless, they permit comparison with the results of others using the same method and the marrow of the lumbar vertebra.

The results, together with other appropriate data, are listed in Table II. The megakaryocytes in the controls varied from 77 to 208 per 100 microscopical fields, or 1791 to 4837 per cmm. of marrow.

In order to determine the cellular pattern of the megakaryocytes, differential counts were done, each based on 200 of these cells. The marrows were prepared according to the modified Wright technic. Only those considered to be satisfactory from a technical standpoint were used. The preparations from 5 of the 16 control cases and from 2 other additional patients, dying of traumatic injuries within 12 hours

TABLE II
Data on Control Series

Number of case	Sex	Age	Duration	Diagnosis	Megakaryocytes per 100 microscopical fields	Megakaryocytes per cmm. of marrow
1	F	55	1 hour	Traumatic injuries	112	2605
2	F	Adult	3 hours	Traumatic injuries	140	3256
3	M	55	1 hour	Traumatic injuries	77	1791
4	M	51	2 hours	Traumatic injuries	146	3326
5	M	33	6 hours	Traumatic injuries	121	2814
6	F	Adult	1 hour	Traumatic injuries	92	2140
7	F	70	1 hour	Traumatic injuries	91	2116
8	M	73	12 hours	Traumatic injuries	93	2162
9	F	42	?	Traumatic injuries	136	3160
10	M	19	3 hours	Traumatic injuries	208	4837
11	M	Adult	3 hours	Traumatic injuries	131	3047
12	M	33	10 minutes	Traumatic injuries	81	1884
13	F	67	6 hours	Traumatic injuries	173	4023
14	M	65	3 hours	Traumatic injuries	126	2930
15	M	Adult	1 hour	Traumatic injuries	78	1814
16	M	60	2 hours	Traumatic injuries	88	2046

of injury, were satisfactory. These included 3 of vertebral pulp and 4 of active humeral marrow. The classification used was similar to that of Custer and Krumbhaar [21] and Nickerson and Sunderland,[3] the exact terminology of the latter being employed. These authors divided the megakaryocytes into three types: (1) young megakaryocytes; (2) adult megakaryocytes; (3) degenerate megakaryocytes. The young form is a large, moderately basophilic cell with a nucleus that is oval or shows early nuclear polymorphism. The nucleus has a characteristically arranged heavy chromatin network. Its cytoplasm is relatively scanty in amount and granularity is absent. The adult megakaryocyte presents a complicated, more or less vesicular nucleus with abundant cytoplasm. It is filled with fine reddish violet to reddish blue granules.[19,22] A hyaline marginal zone may or may not be present. Pseudopods are frequent. The cytoplasmic margins are at times frayed. The degenerate cell is characterized by a shrunken and faded or pyknotic nucleus. The cytoplasm stains poorly and granularity is absent. Typical megakaryocytic nuclei without cytoplasm, or naked nuclei, were

included in the degenerate group. Those cells with well preserved cytoplasm with granules, but with some evidence of nuclear degeneration, were arbitrarily classified as adult megakaryocytes.

The results are tabulated in Table III. In the 7 control cases the cellular pattern of the megakaryocytes did not vary markedly. The young cells constituted 3 per cent and less, the adult ones 71 to 85 per cent and the degenerate ones 13 to 28 per cent of the number of megakaryocytes present.

TABLE III

Cellular Pattern of the Megakaryocytes of the Control Cases

Number of case	Bone marrow	Megakaryocytes			Unclassified
		Young	Adult	Degenerate	
		(%)	(%)	(%)	(%)
7	Vertebral	0	78	20	2
9	Humeral	0	71	28	1
10	Humeral	1	85	13	1
13	Vertebral	2	74	23	1
14	Humeral	3	72	23	2
	Humeral	0	72	27	1
	Vertebral	0	72	28	0

Because of the results to follow in the cases of pneumonia, it is necessary to emphasize certain histological and cytological findings of the marrow of the control series. In the vertebral marrow there was considerable variation in the relative area of tissue occupied by hemopoietic cells and fat cells. Those of the 16 control cases showed a variation ranging from one-fourth or one-third of the area of the sections to be occupied by hemopoietic cells (case 3, Table II; Fig. 8) to three-fourths of the area to be occupied by hemopoietic cells (case 10, Table II; Fig. 9). The entire range of variation was present not only in the sections of marrow from the different cases but in different parts of the same section from certain cases. Not infrequently small isolated fatty foci (Fig. 10) were found in an otherwise active vertebral marrow of the above described limits. It is to be noted that a degree of cellularity which would be normal for case 10 would be gross hyperplasia for case 3. The difficulty of detecting moderate hyperplasia in individuals, such as in case 3, on the basis of degree of cellularity alone is apparent. This brings out the need for a diagnostic criterion of hyperplasia in addition to that of increased hemopoietic cellularity.

An outstanding histological landmark of the vertebral marrow from all 16 control cases was the presence of well defined islands of closely packed erythropoietic cells. These islands averaged 3 to 8 in number per high-power field (Zeiss objective 40 and 7x eyepiece). They were

quite well defined at low magnification, appearing as closely grouped, small, dark nuclei (Figs. 8 and 9).

As previously described, the marrow of the upper third of the humerus, in contrast to that of the lumbar vertebrae, showed an extreme degree of variation in hemopoietic cellularity (Figs. 6 and 7). The size and number of islands of erythropoiesis were likewise quite variable in the active humeral marrows. They varied from less than 1 per high-power field (as in case 15, Table II; Fig. 11) to numerous islands per high-power field (as in case 16, Table II; Fig. 12). There were various intermediate grades of erythropoiesis.

The cellular pattern or qualitative type of hemopoiesis both in the vertebral and active humeral marrows, in contrast to the varying degree of cellularity, was quite constant. The predominant cells of erythropoiesis were the normoblast and the erythroblast in a late stage of maturation. Earlier forms of erythroblasts were infrequent. Myeloblasts were likewise rare but were occasionally found widely scattered. Small round cells and plasmalike cells, scattered widely and occasionally occurring in groups of two or three, were present. Lymphoid nodules were observed. These have been discussed in a previous report.[28] Granulopoiesis was made up almost exclusively of neutrophilic myelocytes C (Fig. 13). Young neutrophilic myelocytes, or myelocytes A and B, which have varying degrees of basophilia and varying numbers of granules in the cytoplasm according to their maturity, were not a prominent feature of the picture. Up to 30 eosinophils per oil immersion field (Zeiss objective 90 and 7x eyepiece) were noted. Metamyelocytes and polymorphonuclear leukocytes were infrequent in this postmortem bone marrow. Rare and widely scattered basophilic myelocytes were present.

Cases of Pneumonia

Megakaryocytes in Pneumonia

The megakaryocytes per 100 microscopical fields were counted and the number per cubic millimeter calculated, as in the control cases. The data are recorded in Table IV. Of the 39 cases of pneumonia, 19, or 49 per cent, showed an increase of megakaryocytes, that is, above 5,000 per cc. of vertebral marrow. Four showed a value below normal and the remainder fell within the range of the controls.

It is appropriate at this point to evaluate the accuracy of the diagnosis of hyperplasia of megakaryocytes by inspection of the vertebral marrow as done in the preliminary data. In Table IV are listed these results as determined by inspection in the 39 cases of pneumonia. They agreed with those obtained by actual counting of the megakaryo-

TABLE IV

Data on Cases of Pneumonia

	Number of case	Megakaryocytes in 100 microscopical fields*	Megakaryocytes per cmm. of vertebral marrow†	Megakaryocytic hyperplasia by inspection of vertebral marrow	Leukoblastic hyperplasia of vertebral marrow	Cellular pattern of granulopoiesis; group	Evaluation of humeral marrow	Remarks
A. Cases showing no increase of megakaryocytes	1	63	1465	Absent	Doubtful		Active	None
	2	122	2837	Absent	Absent		Fatty	None
	3	161	3744	Absent	Absent		Active	Shift to left in erythropoiesis in vertebra; no anemia
	4	207	4814	Absent	Absent		Fatty	None
	5	202	4698	Absent	Absent	IV	Active	None
	6	128	2977	Absent	Absent	III	Active	None
	7	123	2860	Absent	Absent		Fatty	None
	8	132	3070	Absent	Absent	III	Active	None
	9	51	1186	Absent	Absent	II	Active	None
	10	39	907	Absent	Absent	III	Active	Unclassified hyperplasia in vertebra; anemia; RBC 1.62 million
	11	132	3069	Absent	Absent	II	Active	None
	12	122	2837	Absent	Absent	I	Active	Cellular pattern of granulopoiesis in humerus, group II
	13	207	4814	Absent	Absent		Fatty	None
	14	93	2163	Absent	Absent	II	Active	None
	15	162	3767	Absent	Present	III	Meg. hyp.‡ and hyp.	None
	16	98	2279	Absent	Absent	I	Hyp.	Unclassified hyperplasia in vertebra; anemia; RBC 2.6 million
	17	137	3186	Absent	Doubtful	III	Active	None
	18	61	1419	Absent	Absent	II	Active	None
	19	90	2093	Absent	Absent		Active	None
	20	201	4674	Present	Present	II	Active	None
B. Cases showing an increase of megakaryocytes	21	289	6721	Present	Present	II	Active	None
	22	305	7093	Present	Present		Fatty	None
	23	238	5539	Present	Present		Fatty	None
	24	241	5605	Present	Present	I	Meg. hyp.	None
	25	250	5814	Present	Present	III	Meg. hyp.	None
	26	347	8069	Present	Present	III	Meg. hyp. and hyp.	None
	27	403	9372	Present	Present	IV	Meg. hyp.	None
	28	249	5790	Absent	Present	III	Meg. hyp. and hyp.	None
	29	227	5279	Present	Present	II	Meg. hyp.	None
	30	263	6116	Absent	Present		Active	None
	31	575	13372	Present	Present	IV	Meg. hyp. and hyp.	None
	32	322	7484	Present	Absent	II	No section	None
	33	342	7953	Present	Present	IV	Fatty	None
	34	265	6162	Absent	Present	III	Meg. hyp. and hyp.	None
	35	263	6116	Present	Present	II	Active	None
	36	220	5116	Present	Present	I	Active	Cellular pattern of granulopoiesis in humerus, group I
	37	229	5326	Present	Present	IV	Active	None
	38	429	9976	Present	Present	I	Fatty	None
	39	337	7837	Present	Present	III	Fatty	None

* Normal, 77 to 208.

† Normal, 1701 to 4837.

‡ Hyp. = hyperplasia; Meg. = megakaryocytic; Active = degree of hemopoietic cellularity within normal range, but disregarding qualitative changes in granulopoiesis.

cytes in 35, or 90 per cent, of the cases. Thus it is seen that the diagnosis of hyperplasia of megakaryocytes by inspection of the vertebral marrow and comparison with adequate controls is a reasonably accurate method in any series of cases. This obviously is a great advantage since it allows for the analysis of a large number of cases in a relatively short time as compared to the more laborious and time-consuming method of enumeration.

The cellular pattern of the megakaryocytes was determined by differential counts of the three types of cells, as in the control cases. Thirteen sections of marrow, including 5 of vertebral pulp and 8 of active humeral marrow, from 12 of the 39 cases of pneumonia were considered technically satisfactory. They were prepared according to the modified Wright technic. Ten of these sections showed a hyperplasia of the megakaryocytes and 3 did not. These differential counts did not vary significantly from those of the controls. The adult megakaryocytes with their complicated nucleus and finely granular cytoplasm constituted 68 to 84 per cent of all these giant cells.

The occurrence of a thrombocytopenia during the febrile period followed by a thrombocytosis during convalescence in acute infectious diseases, including pneumonia, is well known.[24-27] In these cases of pneumonia the presence of normal or increased numbers of megakaryocytes in all except 4 cases, together with a normal cellular pattern of these cells, is evidence that the thrombocytopenia does not depend on changes in the megakaryocytes.

Parallelism of Megakaryocytic and Leukoblastic Hyperplasia

Associated with the increase of megakaryocytes there were certain other changes in the vertebral marrow. These consisted of a marked increase in hemopoietic cellularity with a corresponding decrease in fat cells. This was accompanied by a marked decrease to almost complete absence of well defined, compact islands of erythropoiesis (case 31, Table IV; Fig. 3). Even a lesser degree of cellularity at about the upper limits of normal was always accompanied by a decrease in the size and number of islands of erythropoiesis and in certain cases by almost complete absence of such islands (case 22, Table IV; Fig. 4). Neutrophilic myelocytes in varying stages of maturation, together with fewer metamyelocytes and polymorphonuclear leukocytes, were the predominant cells of granulopoiesis.

The degrees of maturity in granulopoiesis could be divided into four groups for descriptive purposes. No sharp line of demarcation existed between the different groups. In group I there was no variation from the controls (Fig. 13). Group II showed myelocytes C still to be

the predominant cells and there was a slight but definite increase in myelocytes A and B. This group represented a slight shift to the left in granulopoiesis. In group III there was a moderate decrease in myelocytes C and a moderate increase in myelocytes A and B. Myeloblasts were more frequent than usual. Group IV represented a marked shift to the left in granulopoiesis. Myelocytes C were almost completely absent and myelocytes A were the predominant cells (case 33, Table IV; Fig. 14). Also myeloblasts and myelocytes B were increased. Frequently in all groups there was an increase of the small round cells of the bone marrow, which were scattered throughout, and an increase of the plasma cell-like cells. The latter were frequently collected into groups of four or five cells. Neutrophilic metamyelocytes, leukocytes and eosinophils showed no particular change. Differences between the cellular pattern of the vertebral and of the active humeral marrow of the same case were only slight. For example, granulocytopoiesis in the humerus might be classified as group IV while that in the vertebra as group III. In 29 of the 39 cases the marrow preparations were considered satisfactory for this cytological study. The results of the classification of granulopoiesis in the vertebra into the four groups are listed in Table IV. It is seen that these changes in the cellular pattern of granulopoiesis occurred irrespective of the presence or absence of an increase in megakaryocytes or leukoblastic cells in general.

In addition, 2 cases (nos. 10 and 16, Table IV) showed what might be considered as hyperplasia of both erythroblastic and leukoblastic cells. Both of these cases had a severe unexplained anemia. One other case (no. 3) showed a moderate shift to the left in erythropoiesis. This was a typical case of primary pneumonia of 13 days' duration due to type III pneumococcus. There was no anemia. Other than this single case there was no detectable qualitative change in erythropoiesis.

To emphasize the changes in the vertebral marrow associated with an increase in megakaryocytes, leukoblastic hyperplasia may be defined as follows. Leukoblastic hyperplasia is characterized by a degree of hemopoietic cellularity at or above the upper limits of normal and the neutrophilic myelocyte in various stages of maturation is the predominant cell of granulopoiesis. This is accompanied by a change in erythropoiesis varying from a decrease in the size and number of erythropoietic islands to almost complete absence of such.

It is emphasized that in addition to the well recognized criterion of increased cellularity the decrease in size and in number of islands of erythropoiesis is a valuable aid in detecting this leukoblastic hyperplasia. For example, Figure 4 illustrating the vertebral marrow of case 22, Table IV, showed a degree of hemopoietic cellularity that is

at the upper limits of the controls. However, the change from normal cellularity in this case was shown by the almost complete absence of islands of erythropoiesis. I interpret such a case as having a degree of cellularity, prior to the onset of pneumonia, at the lower limit of normal, similar to that of case 3 of the controls, Figure 8. With the occurrence of the disease there has been an increase of leukoblastic cells accompanied by a decrease in the size and number of erythropoietic islands. It was only through the latter change that an increase of leukoblastic cells could be detected. In 9 of the cases of pneumonia the erythropoietic component of the criteria of leukoblastic hyperplasia was the deciding factor in making a positive diagnosis.

Using the above criteria of leukoblastic hyperplasia, the results obtained are tabulated in Table IV. In 34, or 87 per cent, of the cases of pneumonia there was an exact parallelism between megakaryocytic hyperplasia and leukoblastic hyperplasia. In the 20 cases without an increase of megakaryocytes there was also an absence of leukoblastic hyperplasia in 16. Of the 4 exceptions, the lack of parallelism in cases 1 and 17 was doubtful (Table IV). On the other hand, in the 19 cases with hyperplasia of megakaryocytes there was also a leukoblastic hyperplasia in 18. In such an organ as the bone marrow where changes may be very difficult to detect and evaluate, this is a high degree of correlation.

As seen in Table I, 8 of the 9 cases of hyperplasia of megakaryocytes without primary pneumonia showed both leukoblastic and megakaryocytic hyperplasia. In at least 6 of them there was a bacterial infection (Table I). In only 1 of these was pneumonia present in addition, and it was a terminal bronchopneumonia. Thus this hyperplasia of megakaryocytes and leukoblastic cells must be regarded as a type reaction which occurs frequently in primary pneumonia and at least in certain other infectious diseases.

Evaluation of the Humeral Marrow

As pointed out in the control series, the marrow of the upper third of the humerus normally shows a wide range of variability as to both the degree of cellularity and the number and size of the islands of erythropoiesis. This makes it difficult to determine what the picture of this marrow may be before the onset of any particular disease. Only those changes clearly and definitely beyond the wide range of the normal degree of cellularity can be recognized.

The marrow of the humerus in the pneumonia series could be classified into five types in the following manner. (1) Fatty. Examples of this group showed no hemopoietic cellularity. (2) Active. In these

marrows there was active hemopoiesis with the same variable extent and degree of hemopoietic cellularity and erythropoiesis as in the controls. They occurred with and without qualitative changes in granulopoiesis. (3) Megakaryocytic hyperplasia. Here the megakaryocytes were markedly increased focally or diffusely throughout the marrow (case 25, Table IV; Fig. 15). The remainder of the marrow was as in (2). (4) Hyperplasia of the marrow. This marrow showed an increase of hemopoietic cellularity beyond the normal range with no demonstrable increase of megakaryocytes. Only one case fell in this group. (5) This last group consisted of those showing both general hyperplasia and megakaryocytic hyperplasia (case 31, Table IV; Fig. 16). All of these cases showed some degree of shift to the left in granulopoiesis. The islands of erythropoiesis varied from few in number and minute in size to moderate in size and number. The results of this classification are tabulated in Table IV.

Of the 38 cases of pneumonia in which the humeral marrow was studied, 9 showed an increase of megakaryocytes. In only 5 of these was there likewise a demonstrable increase in the degree of hemopoietic cellularity. One would hardly be justified, on the basis of such data from the marrow of the humerus, in concluding that a parallelism exists between megakaryocytic and leukoblastic hyperplasia. In addition to the above 9 cases, study of the vertebral marrow by the comparative method of inspection showed an increase of megakaryocytes in 10 other cases. Study of the humeral marrow alone would have missed these 10 cases. In only 3 cases was an increase of megakaryocytes diagnosed in the humeral and not in the vertebral marrow by inspection. The necessity of studying the vertebral marrow in detecting and interpreting reactions is even more strikingly illustrated by the findings relative to leukoblastic hyperplasia as previously defined. Leukoblastic hyperplasia was demonstrated by the study of the vertebral marrow in 20 cases while hyperplasia in the humerus, as defined, was present in only 6 cases.

For the sake of analysis of the findings in the humeral and vertebral marrows of the same individual, as recorded in Table IV, the cases of the pneumonia series may be divided into four groups, A, B, C and D. The data below were based on the enumeration of the megakaryocytes in the vertebral marrow. Three cases (nos. 1, 17 and 32) were excluded because of insufficient or doubtful findings.

Group A. In 13 cases (nos. 2, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 18 and 19) megakaryocytic and leukoblastic hyperplasia were absent in both the marrow of the vertebra and of the humerus. Cytological studies on 7 of this group showed a greater or lesser degree of shift to the left in

granulopoiesis. There was no essential difference in this respect between the marrows of the two bones. The humeral marrow was active in all 7 of these cases. There were no studies of the cellular pattern in the 6 remaining cases.

Group B. In 6 cases (nos. 22, 23, 33, 36, 38 and 39) there was a leukoblastic and megakaryocytic hyperplasia in the marrow of the vertebra while that of the humerus was entirely normal. In 5 the humeral marrow was fatty. The cellular pattern was normal in 2 cases, one of which had a fatty and the other a normal active humeral marrow. Granulopoiesis was shifted to the left in 2 and not studied in 2 cases.

Group C. Five cases (nos. 20, 21, 30, 35 and 37) showed a leukoblastic hyperplasia and 4 of these had an increase in the megakaryocytes in the vertebrae. These changes were not demonstrable in the associated humeral marrows, all of which were active. There was a shift to the left in granulopoiesis of both bones in the 4 cases so studied.

Group D. In 8 cases (nos. 24, 25, 26, 27, 28, 29, 31 and 34) there was a leukoblastic and megakaryocytic hyperplasia in the vertebra and a hyperplasia of the marrow or increase in megakaryocytes, or both, in the humerus. A shift to the left in granulopoiesis was present in 7 cases and absent in 1 case. There was agreement in this respect between the marrows of the two bones in the same individual.

The presence of leukoblastic and megakaryocytic hyperplasia in the vertebra associated with a normal humeral marrow in the 6 cases of group B, plus the presence of such hyperplasia in the marrow of both bones in the 8 cases of group D, permits the concept that these changes begin first in the vertebrae and only later take place in the diaphysis of the humerus. The cases of groups A and C are in agreement with this concept.

The data on the qualitative changes show that when there is a shift to the left in granulopoiesis the changes in the two bones are similar provided that there is activity of the humeral marrow. If the humeral marrow is normally fatty in any individual case, the shift to the left would begin in the vertebrae since there would be no hemopoiesis in the diaphysis of the humerus at first. This possibility is exemplified by the 5 cases of group B. However, the constant finding of similar qualitative changes in granulopoiesis in both bones of the same individual when the humeral marrow was active indicates that red marrow, wherever present, functions in this respect at or about the same time. This does not deny the concept that an increase above the normal level in megakaryocytes and leukoblastic cells in general takes place first in the vertebrae and later in the humerus.

Of the remaining cases not included in this summary, 2 (nos. 12 and 15) are possible but not conclusive exceptions to the concept (Table IV). The other 2 cases (nos. 10 and 16) with their unclassified hyperplasia are not exceptions to the rule.

Miscellaneous

The material for this report was collected prior to the advent of treatment of pneumonia with the sulfonamide drugs. Two cases only (nos. 13 and 32) were treated with prontylin. Antipneumococcus serum was administered to 9 of the 39 cases. Seven of these showed hyperplasia of megakaryocytes. However, 12 cases which had received no serum therapy showed an increase of the megakaryocytes. Leukocytic counts on the blood were available in 18 of the 39 cases. Four cases showed 3,000 to 5,000 neutrophilic polymorphonuclear leukocytes per cubic millimeter. In 3 of these there was a moderate to marked shift to the left in granulopoiesis and the cellular pattern was normal in the other case. The remainder of the 18 cases showed a higher leukocytic count.

Summary

On the basis of inspection of the vertebral marrow and comparison with the controls, megakaryocytic hyperplasia was demonstrated in 26 of 306 unselected cases. Primary pneumonia occurred in 39, or 12 per cent, of the unselected cases whereas it occurred in 17, or 65 per cent, of the cases of megakaryocytic hyperplasia. This shows a definite relationship between pneumonia and hyperplasia of megakaryocytes. Counts of the megakaryocytes in the 39 cases of primary pneumonia revealed a number above the normal, *i.e.*, above 5,000 per cmm. of vertebral marrow, in 19 cases. There was no significant variation from the normal in the relative numbers of the types of megakaryocytes. Inspection of the marrow is a reliable method for the determination of an increase in the megakaryocytes in any series of cases.

In pneumonia there is a striking parallelism between hyperplasia of megakaryocytes and of leukoblastic cells. This megakaryocytic and leukoblastic hyperplasia occurred in infectious diseases in the absence of primary pneumonia and it is to be regarded as a type reaction. The hyperplasia begins first in the marrow of the lumbar vertebrae and only later takes place in that of the diaphysis of the humerus.

The study of the marrow of the vertebrae in contrast to that of the humerus or other long bones is necessary in order to detect and classify the reactions of the marrow.

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DESCRIPTION OF PLATES

PLATE 167

FIG. 1. Adult megakaryocytes of the rabbit. The cytoplasm is finely granular. There is a hyaline marginal zone. Wright's technic. × 570.

FIG. 2. Adult human megakaryocyte. There is a finely granular cytoplasm and a hyaline marginal zone. Modified Wright's technic. × 570.

FIG. 3. Pneumonia series. Case 31. Vertebra. A marked increase of megakaryocytes and of other hemopoietic cells is present. There is absence of definitive islands of erythropoiesis such as appear in Figures 8 and 9. Hematoxylin and eosin stain. × 100.

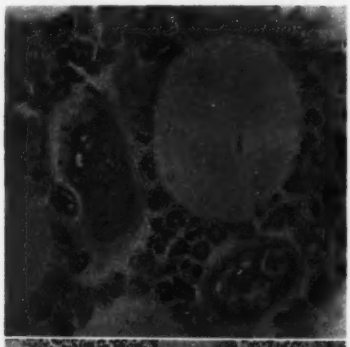
FIG. 4. Pneumonia series. Case 22. Vertebra. There is a moderate increase of megakaryocytes. The degree of hemopoietic cellularity is near the upper limit of normal. Well defined islands of erythropoiesis are absent in contrast with the marrow shown in Figures 8 and 9. Hematoxylin and eosin stain. × 100.

FIG. 5. Pneumonia series. Case 27. Humerus. A megakaryocyte with a bizarre mitotic figure. Hematoxylin and eosin stain. × 500.

FIG. 6. Control series. Case 12. Humerus. The marrow is completely fatty. The cells present are erythrocytes. Hematoxylin and eosin stain. × 100.

FIG. 7. Control series. Case 1. Humerus. This represents the maximum normal degree of hemopoietic cellularity for this location. Four megakaryocytes are present. Hematoxylin and eosin stain. × 100.

FIG. 8. Control series. Case 3. Vertebra. About one-fourth of the area is occupied by hemopoietic cells. Islands of erythropoiesis, composed of closely grouped cells with small black nuclei, are prominent. Hematoxylin and eosin stain. × 100.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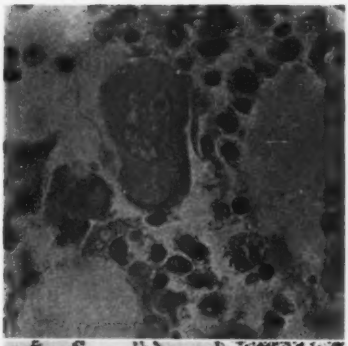
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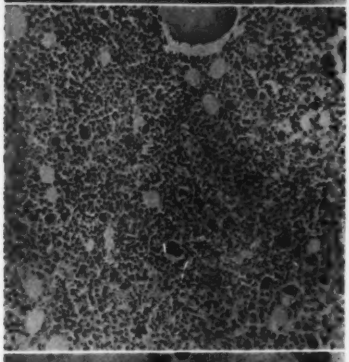
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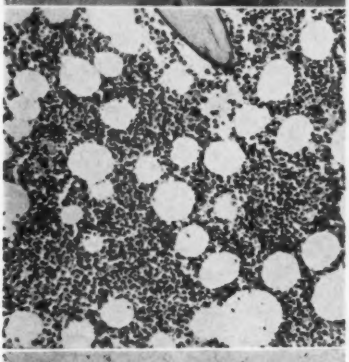
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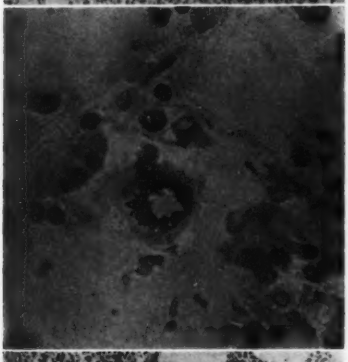
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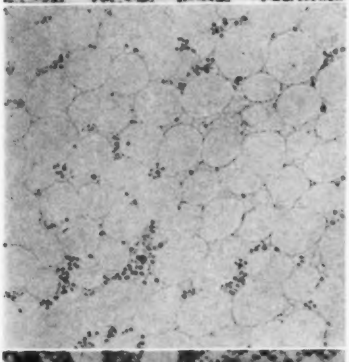
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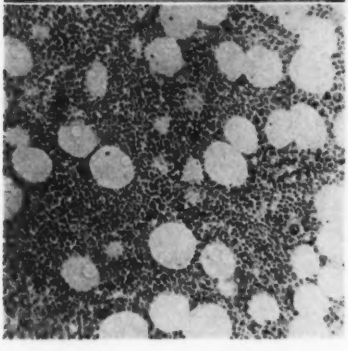
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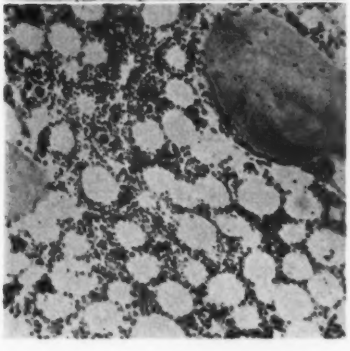
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PLATE 168

FIG. 9. Control series. Case 10. Vertebra. About three-fourths of the area is occupied by hemopoietic cells. This is the upper limit of normal. The islands of erythropoiesis identified as closely packed small black nuclei are frequent and well defined. Hematoxylin and eosin stain. × 100.

FIG. 10. Control series. Case 12. Vertebra. A fatty focus in an otherwise active marrow. Hematoxylin and eosin stain. × 100.

FIG. 11. Control series. Case 15. Humerus. The islands of erythropoiesis are few in number and are ill-defined. Hematoxylin and eosin stain. × 100.

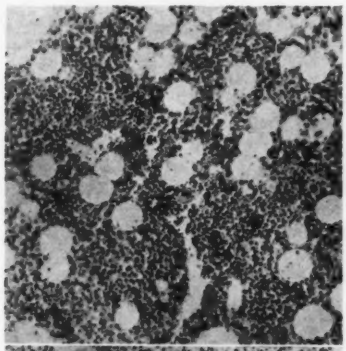
FIG. 12. Control series. Case 16. Humerus. The islands of erythropoiesis, composed of closely packed cells with small black nuclei, are frequent and prominent as compared with Figure 11. Hematoxylin and eosin stain. × 100.

FIG. 13. Control series. Case 6. Vertebral pulp. The predominant cell of granulopoiesis is the mature neutrophilic myelocyte, which is identified by its cytoplasm being packed with fine granules. Azure II and eosin stain. × 777.

FIG. 14. Pneumonia series. Case 33. Vertebral pulp. The predominant cell of granulopoiesis is myelocyte A. Its cytoplasm either lacks granules or contains only a few as compared with the mature myelocytes shown in Figure 13. Azure II and eosin stain. × 570.

FIG. 15. Pneumonia series. Case 25. Humerus. The degree of cellularity in general is within the range of the controls. There is an increase in megakaryocytes as compared with the normal marrow of Figure 7. Hematoxylin and eosin stain. × 100.

FIG. 16. Pneumonia series. Case 31. Humerus. There is a marked increase in the total cellularity and in the number of megakaryocytes. Hematoxylin and eosin stain. × 100.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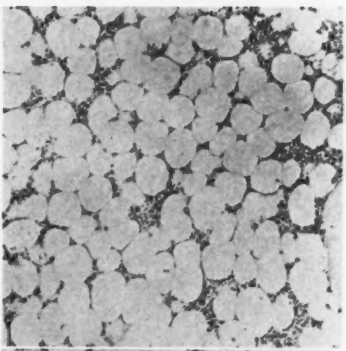
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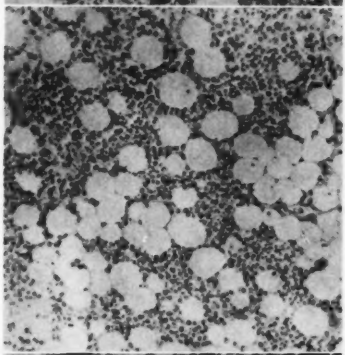
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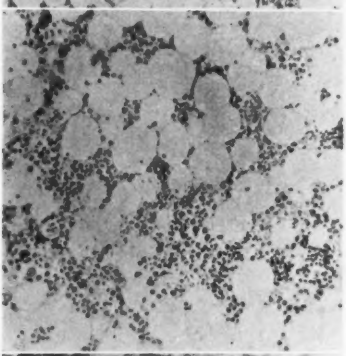
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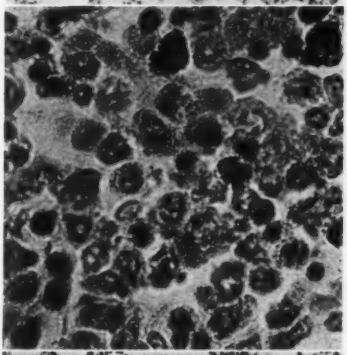
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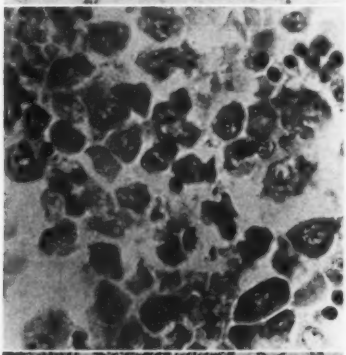
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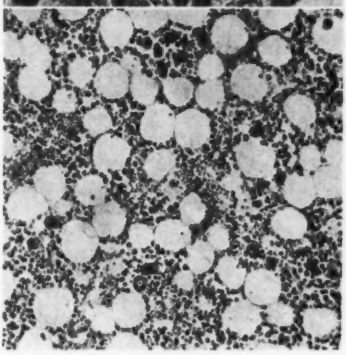
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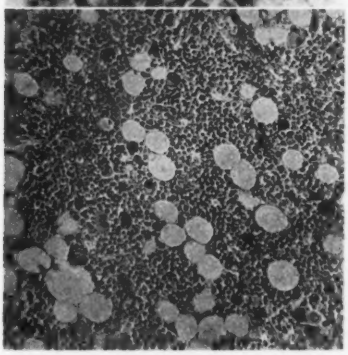
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STUDIES ON FOWL LEUKOSIS *
TRANSFER TO THE CHICK EMBRYO

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The purpose of this paper is to report experiments with fowl leukosis, planned to determine: (1) whether fowl leukosis could be transmitted to the embryo by inoculation of the chorioallantoic membrane of the developing egg with blood, tissue and tissue filtrates of affected fowls, and (2) whether the agent of fowl leukosis can be cultivated and intensified by growth on the membrane of the developing egg.

Attempts at cultivation of the virus of fowl leukosis have been made by a few investigators. The tissue-culture technic for cultivation of virus was used by Furth and Stubbs (1934), Verne, Oberling and Guérin (1936) and Furth and Breedis (1937). In these experiments leukotic tissues were incubated in Carrel flasks, for varying periods of time, and the presence of the virus tested by inoculation of fowls with the cultures. Each of these observers concluded that, although the virus could be maintained for a short period while the growth of leukotic cells was maintained, cultivation of the agent had not been obtained.

Ruffilli (1937) was the first observer to claim that the virus could be cultivated in cultures of fibroblasts from heart muscle, without the presence of hematopoietic cells. Doljanski and Pikovski (1940) reported a small number of experiments which will bear confirmation since they are at variance with more extensive investigations. They reported successful takes in young fowls inoculated with tissue culture of embryonic heart muscles, bathed for 1 hour in a Berkefeld filtrate of leukotic spleen and subsequently grown in chick plasma in Carrel flasks. The cultures were incubated 2 and 4 weeks; of 16 chickens inoculated with these cultures, 15 were reported to have developed leukosis 12 and 20 days later. They also reported successful takes among fowls inoculated with washings of the original culture, maintained by transfer for 86 days; the washings produced leukosis in inoculated fowls even after the culture had undergone fibroblastic transformation.

At the time the studies reported in his paper were started, Jármai (1935) was the only investigator who had attempted to transmit leukosis to chicks by inoculating the egg with the virus. His attempts were successful only when eggs were inoculated after the ninth day of incu-

*This study was made possible by grants from the Elizabeth McCormick Fund of the Institute of Medicine of Chicago, the Edward Shedd Wells Memorial Fellowship Fund of The Children's Memorial Hospital, and the Otho S. A. Sprague Memorial Institute, Chicago.

Received for publication, February 2, 1942.

bation. He concluded that, since bone marrow appeared at this time, the virus required bone marrow tissue for maintenance.

Since then, Kirschbaum, Stern and Hooker (1940) reported successful takes in 3 of 21 fowls 45 and 47 days old, which were inoculated with a Chamberland filtrate of leukotic plasma on the twelfth day of incubation of the developing egg. They presented no data regarding the actual cultivation of the virus. Using unfiltered plasma as the inoculum, they observed leukosis in chicks inoculated on the seventh day of the developing egg.

Storti and Mezzadra (1940) reported the transmission of leukosis to the embryo when the chorioallantoic membrane was inoculated with cell suspensions of leukotic tissue as early as the fourth day of development. They transmitted the disease to fowls by intramuscular inoculation of the embryonic tissue, of the first transmission, but were unable to prove the presence of the virus by similar inoculation of fowls after transmission to the second egg. They concluded that the virus could be maintained but not cultivated. However, since cell suspensions were used as the inoculum in their experiments, conclusions regarding the transmission of the virus must be guarded.

Method

A strain of leukotic fowl was developed for this experiment from leukotic fowls kindly supplied by Dr. Jacob Furth. Intravenous injections of leukotic blood or plasma into successive batches of young chickens maintained the strain. The developing egg technic, used by Rous and Murphy (1911) for study of fowl tumors, by Danchakoff (1920) for investigation of the effect of spleen grafts on the mesenchymal tissue of the embryo, and recently by Goodpasture and Anderson (1937), Burnet (1936) and numerous other investigators for the cultivation of viruses, was employed for the embryo experiments.

Fertile eggs were incubated at 39.5° C. for 9 to 11 days. A window 1.0 cm. square was then made in the shell with a dental drill, exposing the chorioallantoic membrane. The tissue or filtrate to be cultured was then placed on the membrane, the window rimmed with vaseline and sealed with a coverslip. The eggs were then reincubated for a variable number of days. Observations for gross change in appearance of the membrane were made through the coverslip; membranes and embryos were inspected for gross change before fixation in formaldehyde-Zenker's solution for histological study. Both membrane and embryos were sectioned and stained with hematoxylin and eosin.

The criteria of leukemic change in the embryo were: (1) intravascular and extravascular accumulations of hemocytoblasts; (2) replacement of the normal cellular content of the bone marrow by hemocyto-

blasts, and (3) leukemic proliferation of the perivascular mesenchyme. Uninoculated embryos and embryos inoculated with normal chick leukocytes and spleen pulp served for controls.

Criteria of leukemic change in the older chickens were: (1) pale comb, (2) hemocytoblastosis and (3) characteristic leukemic infiltration in the organs.

I. Transmission to the Embryo with Spleen Pulp, Blood Leukocytes, Unfiltered Plasma and Berkefeld Filtrates of Spleen Extracts and Plasma

A. Transmission Experiments with Viable Cells from Spleen Pulp and Blood

Experiment 1. Spleens of leukotic fowls were removed aseptically, minced in Tyrode's solution, cultured for sterility and a small bit, 2 mm. in diameter, explanted onto the chorioallantoic membrane. In 45 per cent of the embryos examined after 20 days of incubation leukemic changes were found in the organs.

Table I

Percentage of Takes in Embryos Inoculated with Leukotic Spleen Pulp

Test number	Age of embryo when inoculated	Number of eggs inoculated	Number alive on 20th day of incubation	Embryos showing pathological changes of leukosis	Percentage of takes among embryos surviving experiment
1	10 days	12	3	1	33%
2	9 days	19	2	1	50%
3	10 days	16	3	3	100%
4	9 days	12	5	1	20%
5	9 days	12	1	1	100%
6	13 days	14	3	1	33%
Total		85	17	8	47%

Experiment 2. Leukocytes from the buffy coat of heparinized blood drawn from leukotic fowls were pipetted directly onto the chorioallantoic membrane. In two experiments all embryos which survived the incubation period showed characteristic leukemic changes in the liver, spleen and bone marrow.

Table II

Takes in Embryos Inoculated with Leukocytes of Leukotic Fowls

Test number	Age of embryo when inoculated	Number of eggs inoculated	Number of embryos alive on 20th day of incubation	Number of embryos fixed for histological study	Number of embryos allowed to hatch	Takes			
						Embryos		Hatched chickens	
						no.	%	no.	%
8	10 days	8	4	3	1	3	100	1*	100
11	9 days	11	2	2	0	2	100		
Total				5	1	5	100	1	100

* Died of leukosis at age of 5 weeks; blood leukocyte used for experiment.

B. Transmission to the Embryo and to Older Chickens by Unfiltered Plasma

Experiment 3. Plasma was prepared by centrifugating leukotic blood at 6000 r.p.m. for 10 minutes. Two to 6 drops of the undiluted plasma were pipetted onto the chorioallantoic membrane. Five of 19 embryos which survived the 21-day period of incubation showed signs of leukosis.

TABLE III

Percentage of Takes in Eggs Inoculated with Plasma

Test number	Age of embryo	Number inoculated	Number of embryos alive on 20th day	Number of embryos fixed on 20th day	Number of embryos hatched	Takes			
						Embryos		Chickens hatched	
						no.	%	no.	%
7	10 days	8	6	3	3	0	0		0
9	10 days	10	4	2	2	0	0	1	50%
10	10 days	8	3	3	0	2	66%	0	0
11	11 days	12	3	2	0	2	100%	0	0
12	9 days	15	4	4	0	0	0	0	0
Total				14	5	4		1	

Total number of embryos alive at end of incubation period, 19.
Number and percentage of takes among embryos alive at end of incubation period, 4 (28%).
Number and percentage of takes after hatching, 1 (20%).

Experiment 4. Older chickens inoculated intravenously with undiluted plasma developed signs of leukosis more regularly than did the embryos, although in the individual experiments, when embryos survived the period of incubation, the percentage of takes was comparably high.

TABLE IV

Percentage of Takes in Older Chickens Inoculated with Plasma

Test number	Preparation of plasma	Dose	Age of chickens	Number inoculated	Number of takes	Takes	Incubation period
2	Centrifugated	0.5 cc.	6 weeks	4	3	74%	16, 40, 43 days
11*	Centrifugated	0.1 cc.	5 days	5	5	100%	29, 58, 76, 88 days
12	Centrifugated	0.1 cc.	2 weeks	4	1	25%	28 days
720	Centrifugated	0.2 cc.	3 weeks	4	2	50%	28 days
21	Centrifugated	1.0 cc.	6 weeks	4	1	25%	8 weeks
23	Centrifugated twice	0.5 cc.	6 weeks	3	1	33%	5 weeks
27a	Centrifugated	0.5 cc.	6 weeks	2	2	100%	26 days
5	Serum	0.5 cc.	6 weeks	4	0	0	.....
10	Serum	0.5 cc.	6 weeks	3	1	33%	5 weeks
Total				33	16	48%	

* Plasma produced typical leukosis in 4 of 5 five-day-old chickens.

Experiment 5. In three experiments, 16 embryos were allowed to hatch. One of the 16 developed leukosis after 5 weeks.

TABLE V

Leukosis Developing in Chickens Inoculated as Embryos with Leukotic Plasma

Test number	Inoculum	Age of embryo when inoculated	Number inoculated	Number hatched	Number of chickens developing leukemia	Inoculation period
9	Undiluted plasma	10 days	8	3	1*	5 weeks
16	Plasma diluted 3:1, filtered through Berkefeld V	10 days	12	6	0	.....
17	Plasma diluted 4:1, filtered through Berkefeld V	10 days	12	7	0	.....
Total			32	16	1	

* Plasma of this chicken transmitted leukosis to 4 of 5 chickens inoculated intravenously with 0.1 cc. of undiluted plasma, and 2 of 12 embryos inoculated on ninth day of incubation showed leukotic change in liver and bone marrow after 21 days of incubation.

C. Transmission Experiments with Berkefeld Filtrates of Spleen and Plasma

Experiment 6. Filtrates of tissue of leukotic spleen were prepared by passing a suspension of spleen pulps in Tyrode's solution through a gauze filter and then through a Berkefeld V filter. Cultures for sterility were made before and after filtration. The chorioallantoic membrane was inoculated with 0.5 to 1.0 cc. of the filtrate. In no instance was leukosis transmitted to the embryo by these filtrates.

TABLE VI

Results of Inoculation of the Chorioallantoic Membrane with Berkefeld Filtrates of Leukotic Spleens

Test number	Inoculum	Age of embryo when inoculated	Number of eggs inoculated	Number of embryos alive on 21st day	Embryos showing histological changes typical of leukemia
14	Splenic filtrate	9 days	24	6	0
15	Plasma filtrate	9 days	18	2	0
16	Plasma filtrate	9 days	12	8 (2 fixed)	0 (6 embryos hatched)
17	Plasma filtrate	10 days	12	7 (2 fixed)	0 (5 embryos hatched)
20	Splenic filtrate	10 days	10	2	0
21	Splenic filtrate	10 days	10	2	0
24	Splenic filtrate	11 days	12	3	0
Total				30	0

Experiment 7. Twenty-five per cent of the older chickens inoculated intravenously with these spleen filtrates developed leukosis.

TABLE VII

Percentage of Takes in Older Chickens Inoculated Intravenously with Berkefeld Filtrates of Leukotic Spleen and Plasma

Test number	Inoculum	Chickens		Takes		Incubation period
		Age when inoculated	Number inoculated	Number	%	
13	Splenic filtrate	3 weeks	4	2	50%	12 days, 10 weeks
15	Plasma filtrate	4 weeks	4	1	25%	10 days
17	Plasma filtrate	6 weeks	3	0	....	.....
20	Splenic filtrate	3 weeks	4	2	50%	6 weeks, 4 months
24	Splenic filtrate	6 weeks	3	0	....	
31	Splenic filtrate	6 weeks	4	1	25%	4 months
32	Splenic filtrate	6 weeks	2	0	....	.....
Total			24	6	25%	

II. Transmission to Chicks by Inoculation of Pooled Membranes Previously Inoculated with Filtrates of Leukotic Tissue

In six experiments membranes were inoculated with Berkefeld filtrates of spleen pulp or plasma; in three instances young chicks were also inoculated intravenously with these same filtrates, to test the

TABLE VIII

Chickens Inoculated with Splenic Filtrates, and with Membranes Inoculated with These Same Filtrates

Experiment number	Inoculum	Takes among fowls inoculated directly with filtrate		Period of incubation after membranes were inoculated	Takes among fowls inoculated with saline suspension of membranes	
		No. inoculated	Takes		No. inoculated	Takes
22	Berkefeld filtrate of spleen–643	6	1	6 days	3	0
25	Berkefeld filtrate of plasma–644	none		10 days	3	1
26	Berkefeld filtrate of spleen no. 23	2	0	6 days	3	0
27	Berkefeld filtrate of plasma–648	2	1	8 days	2	0
33	Berkefeld filtrate of plasma–159	none		48 hours	3	0
34	Berkefeld filtrate of plasma–196	none		5 days	3	0
				4 days	5	1
Total		10	2		22	2

infectivity of the filtrate. After incubation of the filtrate on the membrane for 4 to 6 days, the membranes were removed aseptically, pooled in Tyrode's solution, minced and ground, centrifugated for 5 minutes and 1 cc. of the supernatant fluid injected intravenously into another group of chicks. Evidence that the infectivity of the filtrates was increased by incubation on the membrane was not obtained.

Discussion

In the foregoing experiments, unfiltered suspensions and plasma of leukotic fowls produced leukosis in embryos and in chicks hatched from these embryos when placed on the chorioallantoic membrane after the eighth day of incubation of the egg. Berkefeld filtrates of leukotic tissue and plasma failed to produce the disease in eggs similarly inoculated. Failure of the filtrates to induce the disease in embryos or hatched chicks was not due to the noninfectivity of the filtrates, since they produced leukosis when injected intravenously into young fowls.

Failure of the filtrates to induce the disease in embryos might be due to (1) dilution of the agent in the process of filtration; (2) unsuitability of the chorioallantoic membrane for maintenance of the virus; (3) immunity of the embryo to certain concentrations of the virus. Since no evidence was obtained that the virus had been cultivated on the membrane, it is likely that the concentration of virus reaching the embryo was less than that provided by intravenous inoculation of the filtrate in young fowls. It is possible that the epithelium of the chorioallantoic membrane provides a barrier, and that intravenous inoculation of the embryo would be more successful. It is noteworthy that in the experiment of Kirschbaum, Stern and Hooker (1940) embryos developed leukosis when inoculated by injection of Chamberland filtrates of plasma into the albumin.

Failure of the virus to grow on the membrane may also be due to the unsuitability of the tissue for growth of the virus, and recalls the experiments of Furth and Stubbs (1934), Furth and Breedis (1937), Verne, Oberling and Guérin (1936), in which these investigators were unable to grow the virus in tissue culture without the presence of hematogenic cells. This controversial issue of the problem is heightened by the recent reports of Doljanski and Pikovski (1940) and Ruffilli (1937) who reported contradictory tissue culture experiments in which the virus did survive after the hematogenous cells had disappeared.

The histology of the membrane was not essentially changed by the presence of the filtrates or plasma. It remained transparent, unthickened and free of the focal opacities common to other virus infections

of the membrane. Necrosis of the epithelium did not occur, inclusion bodies were absent and the cellular reaction was no more pronounced than it was in membranes inoculated with sterile broth or saline (Fig. 1). This is quite different from the histological picture of membranes inoculated with cell suspensions of leukotic tissue, which produce gross thickening and cloudiness of the membrane. The thickening is due to intercellular edema, and the opacity to the marked cellular reaction.

The explanted cells of leukotic spleen readily formed grafts which in some instances hypertrophied to several times the size of the original explant (Figs. 2 and 3). Shortly after explantation, the actively ameboid leukemic cells wandered into the membrane away from the explant and mingled with the normal cellular constituents, *i.e.*, neutrophils, lymphocytes and polyblasts. The leukemic cells in various stages of mitosis and disintegration could be traced into the blood capillaries of the membrane and thence into the embryo to the sinusoids of liver and bone marrow. After 48 to 72 hours, the explant was walled off by polyblasts, canalized by blood vessels and a true graft was established. The process was essentially the same as with normal spleen grafts, described by Danchakoff (1920), except that the hypertrophy of the graft was more extensive and rapid, due to the active proliferation of the leukemic cells.

The explanted leukemic blood leukocytes behaved in a manner similar to those of the spleen pulp, except that they did not form grafts, but were rapidly dispersed throughout the membrane and thence into the sinusoids of the embryo. The chorioallantoic membrane provides a culture medium for the maintenance of the leukemic cells, a certain number of which survive, migrate by ameboid motility throughout the membrane and, via the blood vessels, to the embryo where they set up metastatic foci of cell formation.

That the pathological findings of leukosis in the embryo are entirely due to these transported cells seems likely, but, until the nature of the agent is known, this remains a question. The earliest leukemic change was most easily recognizable in the liver, where clumps of hemocytoblasts were first seen in the smaller sinusoids and among the perivascular cells. In the older embryos, the lesions were more typical and were characterized by large aggregates of stem cells throughout the sinusoids of the liver, by intravascular hemocytoblastosis and by wide zones of stem cells (Fig. 5). The embryos showing the most extensive leukemic change were those inoculated with cell suspensions, but those inoculated with plasma also showed convincing, although less striking, changes in the bone marrow and perivascular mesenchyme (Fig. 6).

Early leukemic changes in the bone marrow were more difficult to

follow than those in the liver and the data are insufficient to prove that the bone marrow was primarily involved. In the embryos inoculated with cell suspensions, the presence of foreign hemocytoblasts in the marrow confused the picture. In those inoculated with plasma, the marrow was predominantly hemocytoblastic by the time characteristic early changes were evident in the liver and before hemocytoblastosis developed.

Summary

Experiments are reported in which leukosis was produced in embryos, as well as in young fowls, by inoculation of the chorioallantoic membrane with unfiltered suspensions of leukotic spleen cells, leukocytes and plasma. Leukosis did not develop in embryos inoculated with Berkefeld filtrates of these tissues, although they were infective for young chicks. Suspensions of the membranes inoculated with the filtrates, and incubated for variable lengths of time, failed to produce leukosis in fowls by intravenous inoculation.

Conclusion

While evidence was not obtained that the virus of chick leukosis can be cultivated on the chorioallantoic membrane of the developing egg, the disease can be transmitted to the chick embryo by inoculation of the egg with tissues of leukotic fowls.

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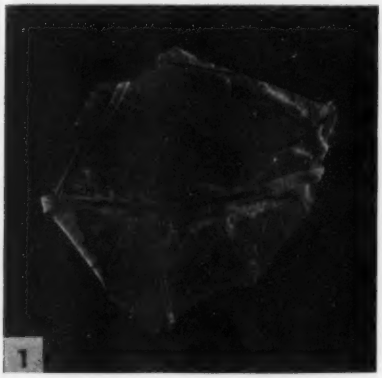
DESCRIPTION OF PLATES

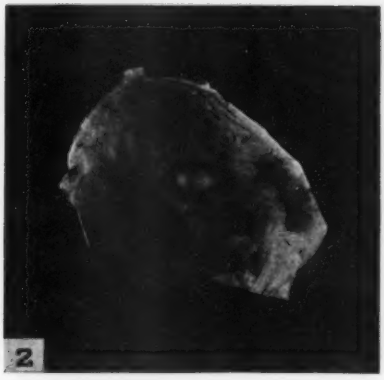
PLATE 169

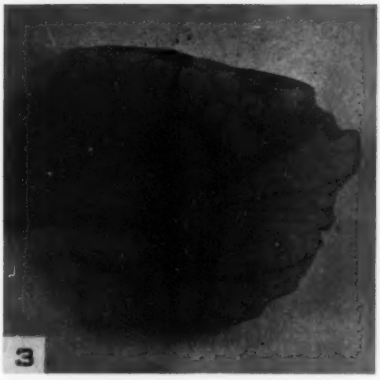
FIG. 1. Photograph of chorioallantoic membrane inoculated on the seventh day of incubation with 10 drops of sterile broth, showing small focal opacities due to collections of inflammatory cells in the mesenchyme.

FIGS. 2 and 3. Photographs of chorioallantoic membranes 9 days after inoculation with minced spleen pulp of leukotic fowl, showing gross hypertrophy of the spleen explant.

FIG. 4. Chorioallantoic membrane inoculated on the 9th day of incubation with leukocytes of a leukotic fowl (no. 3) and fixed for histological examination on the 20th day of incubation. Above and to the right there is a clot of disintegrating explanted leukocytes. The ectoderm is thickened, and there is a cellular reaction within the mesenchyme. × 45.







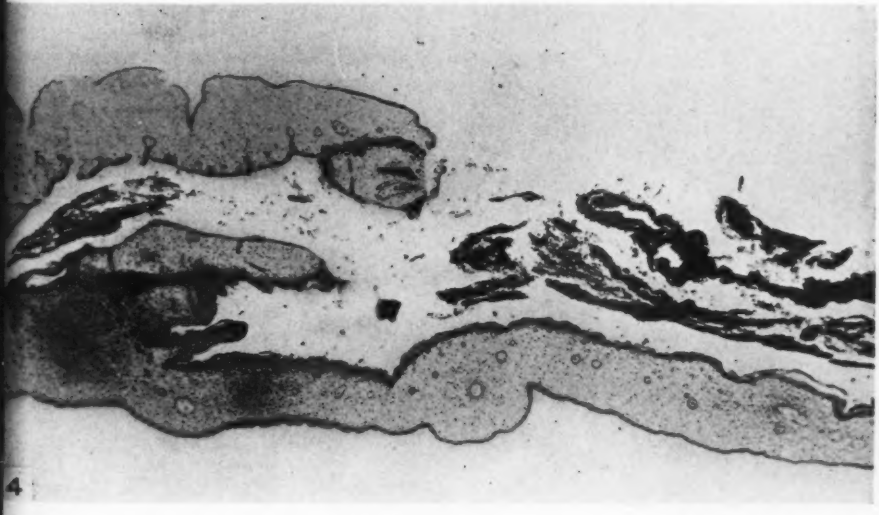
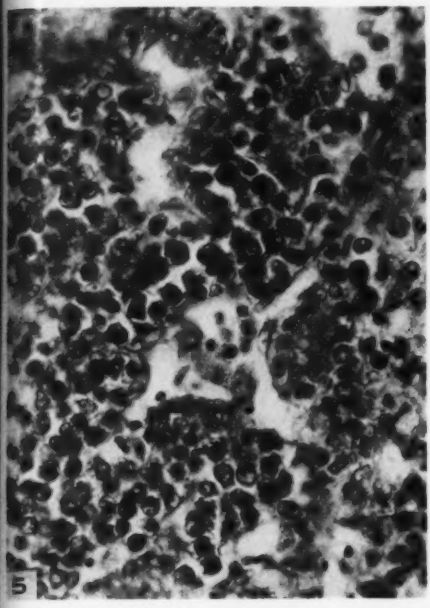


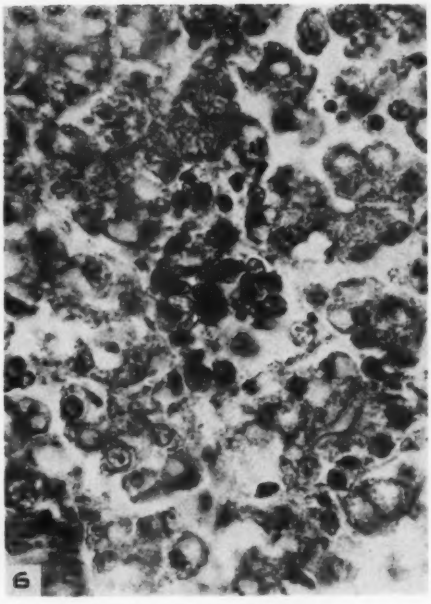
PLATE 170

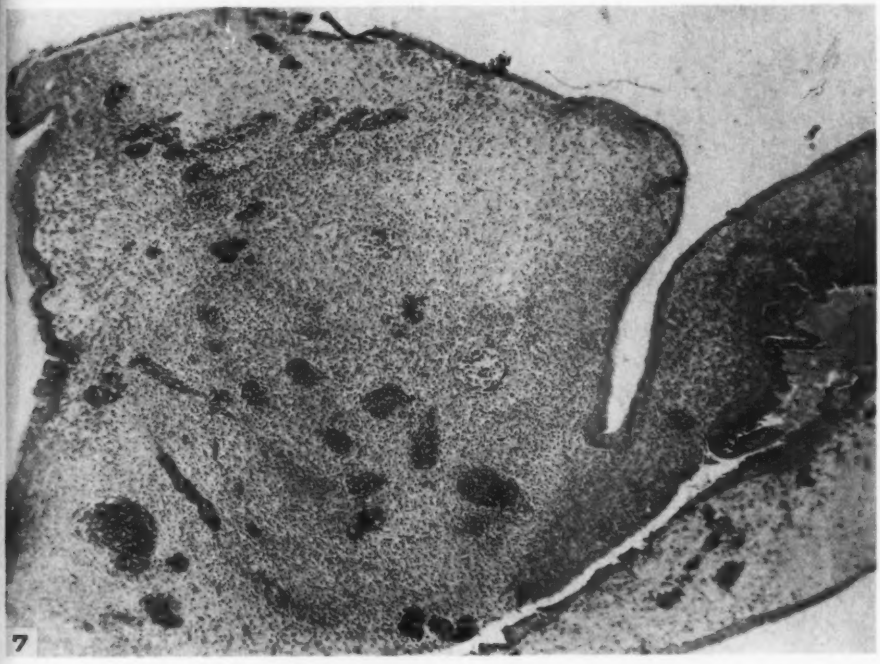
FIG. 5. Liver of an embryo, the chorioallantoic membrane of which was inoculated with leukocytes of a leukotic fowl (no. 3) (see Fig. 4), showing aggregation of stem cells about a central vein and also within the lumen of the vein. × 715.

FIG. 6. Liver of an embryo, the chorioallantoic membrane of which was inoculated with plasma of a leukotic fowl (no. 3) (see Fig. 7), showing a cluster of stem cells within an hepatic sinusoid and vacuolization of liver cells. × 715.

FIG. 7. Chorioallantoic membrane inoculated on the 9th day of incubation with 10 drops of plasma of a leukotic fowl (no. 3), showing edema and cellular reaction in the mesenchymal layer of the membrane. × 70.







EFFECTS OF THE "GROWTH HORMONE" OF THE ANTERIOR HYPOPHYSIS (ANTUITRIN G) ON THE SKELETON OF MICE AND GUINEA PIGS *

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In previous experiments in growing mice and guinea pigs [1-4] we found that acid extracts of the bovine anterior hypophysis, similar to those used by Loeb and Basset [5] in their experiments, induced a temporary stimulation of the proliferation of the epiphyseal cartilage followed by an acceleration and a premature onset of ageing processes in the skeletal tissues. Syngenesiotransplants of mouse anterior hypophysis,[4] on the other hand, caused a temporary inhibition of the growth of the cartilage associated with a prolongation of the growth period. This likewise was followed later by an acceleration of skeletal ageing. In neither case was gigantism observed, nor did the degree of the age changes in the epiphyseal disks surpass the maximum of such changes occurring in normal old animals.

According to Ray, Evans and Becks,[6] Thomson and Collip [7] and some other investigators,[8,9] the anterior hypophyseal gland contains a growth-promoting substance that can be extracted by alkaline, but not by acid solutions. It might, therefore, be assumed that the acid extracts of anterior hypophysis used in our previous investigations did not contain this growth substance. For this reason, we decided to compare the action of an alkaline extract (Antuitrin G) on bone and cartilage of mice and guinea pigs with the action of acid extract employed in our earlier investigations. Antuitrin G is made specifically to obtain a potent preparation of the growth-promoting principle of the anterior hypophysis.

MATERIAL AND METHODS

Forty-three mice and 8 guinea pigs were used in these experiments. One group of mice consisted of growing animals 5 to 6 weeks old at the beginning of the experiments; the other of adult animals, 3 to 4 months old, in which the growth of the cartilage had become very slow or was about to cease altogether. In the first group, 10 mice of strain C_{57} (9 males and 1 female) and 6 of strain D (5 females and 1 male)

* These investigations were carried out with the aid of grants from the International Cancer Research Foundation, from the Jane Coffin Childs Memorial Fund for Medical Research and from the Committee on Research in Endocrinology of the National Research Council.

Received for publication, February 6, 1942.

received subcutaneous injections of 1 rat unit (0.1 cc.) of Antuitrin G three times weekly for periods of 1, 2 and 4 weeks, and 2 and 3 months. In the older group, 6 male mice of strain C_{57} and 6 females of strain D received 1.5 rat units (0.15 cc.) of Antuitrin G three times weekly for periods of 1, 2 and 4 weeks. Nine male mice of the C_{57} strain and 6 females of the D strain of corresponding age served as normal controls. Littermates were used in all groups as far as possible.

Furthermore, 2 female guinea pigs weighing 180 to 200 gm. received daily intraperitoneal injections of 10 rat units (1 cc.) of Antuitrin G for 4 days and 2 others were treated in the same way for 14 days. Four guinea pigs of similar age and weight served as controls.

After necropsy, the endocrine organs, tibia and femur, and, in the case of guinea pigs, the chondro-osseous junctions of several ribs were prepared for histologic study, as described for previous experiments.[2,10]

Observations

In Table I the initial and final weights of each animal are given. Since most of the animals within a group were littermates, the differences within the individual groups may be more indicative of the changes in weight under the given experimental conditions than means based upon a greater number of unrelated individuals.

In the growing mice, the increase of weight during the first and second weeks of the experiments did not surpass that of the controls; there was, on the contrary, rather a slight tendency towards a lesser gain of weight in the treated group. From about the fourth week of treatment this tendency was reversed, and the injected mice gained weight more rapidly than their controls. In the older group, the injected animals showed a greater increase of weight than their controls during the period of observation. Whereas the control guinea pigs gained weight steadily, the injected guinea pigs showed at first a loss, the initial weights being regained after about 2 weeks of treatment.

Histologic Examination

Growing Mice of Strain C_{57} 5 to 6 Weeks Old at the Beginning of the Experiment

After 1 week of injections, the growth zone of the upper tibia was somewhat narrowed, but contained many cartilage cells which were more closely packed in the single rows than usual. The ground substance was diminished. The dispersed nonoriented cartilage cells were quickly converted into columnar cartilage cells which exhibited frequent mitotic division. The layer of hypertrophic cartilage cells was narrower than normal due to an intensified breakdown by capillaries

advancing from the bone marrow and their rapid replacement by osteocytes. In some area, ossification had proceeded so rapidly that unopened hypertrophic cartilage cells became incorporated in the newly formed trabeculae. Regressive changes had set in and amorphous plugs of degenerated cartilage were visible traversing the epiphyseal plate

TABLE I

Weights of the Control Animals and of the Animals Injected with Antuitrin G, in Grams

Growing mice

Duration of experiment	Mice of strain C57				Mice of strain D			
	Controls		Injected		Controls		Injected	
	Init.	Final	Init.	Final	Init.	Final	Init.	Final
1 week	14.5	19.0	14.5	16.3	17.2	19.4	16.0	16.2
			13.5	15.0			16.0	18.7
2 weeks	13.5	15.2	13.0	16.0	12.0	12.6	12.5	16.6
			13.5	14.8			10.0	14.9
4 weeks	13.5	22.5	15.0	21.9	9.0	13.5	9.0	16.2
			15.0	20.8			12.0	18.4
2 months	15.5	22.0	15.0	24.5				
			13.0	24.5				
3 months	16.5	28.0	14.0	27.9				
			12.5	23.2				

Adult mice

Duration of experiment	Mice of strain C57				Mice of strain D			
	Controls		Injected		Controls		Injected	
	Init.	Final	Init.	Final	Init.	Final	Init.	Final
1 week	20.0	22.0	20.5	20.5	19.2	18.5	17.5	20.0
			22.0	28.1			19.3	20.9
2 weeks	20.5	20.5	21.5	24.4	18.8	18.8	19.0	20.0
			21.5	25.3			16.7	19.9
4 weeks	22.0	26.7	22.0	27.7	18.0	18.7	17.5	21.7
	20.5	22.7	23.0	27.7			17.5	22.8

Guinea pigs

Duration of experiment	Controls		Injected	
	Init.	Final	Init.	Final
4 days	175	185	205	172
	175	190	180	177
14 days	198	253	185	193
	175	215	177	176

(Figs. 1 and 2), an occurrence usually not found in healthy mice of the C_{57} strain before the end of the fourth month of life.

In the subepiphyseal layer, the peritrabecular connective tissue cells were converted into epithelioid cells in greater numbers than ordinarily. These latter cells arranged themselves in a beadlike manner along the surface of the spicules and acted as osteoblasts; as a consequence, numerous thick, bony trabeculae were laid down.

The articular cartilage cells underwent an accentuated proliferation associated with increased mitotic division, as well as with intensified hypertrophy and accelerated ossification.

The periosteal and endosteal cells were increased in number, and, in contact with the compacta of the shaft, produced more bone than normal. The vascular canals were narrow, and cellular resorption was less marked than in untreated mice.

After 2 weeks of administration of Antuitrin G, the epiphyseal disk was greatly narrowed and the proliferation of the epiphyseal cartilage had decreased. Calcification and ossification had increased further. Simultaneously, however, processes of resorption acting on cartilage and bone were intensified. There was further narrowing of the epiphyseal growth zone and a thinning and solution of the subepiphyseal trabeculae. In the joint, some cartilage cells had undergone liquefaction.

After 4 weeks of injections of Antuitrin G, the epiphyseal growth zones were distinctly narrower than normal. The cartilaginous matrix and the cartilage cells were heavily calcified. The cells were atrophic, their size being reduced about one-half, but the number of cartilage cells in a single cartilage cell row did not deviate greatly from that of the controls. Proliferation of these cartilage cells was not observed. However, the processes of ossification still appeared accelerated and intensified. The subepiphyseal trabeculae continued to be fairly thin and short and showed transverse links. Thus, in mice 2½ months old, that had been injected for 1 month, a transverse bony plate began to form separating the calcified epiphyseal cartilage from the bone marrow, a condition usually not found in untreated mice of strain C_{57} before the age of 4 to 5 months.

In the articular cartilage, the proliferative and regressive changes receded gradually. In the shaft, the osteocytes were larger than at earlier stages of the experiment and the osseous substance was softer. The vascular canals were enlarged and the cellular resorption of bone by the periosteum was also increased.

After 2 months of injections the epiphyseal disks were narrow and strongly calcified. The cartilage cell columns were short. The persisting cartilage cells, the cartilaginous ground substance and the degenerative plugs, that in the meantime had increased in number and extent, were densely calcified. There was no evidence of proliferation of the cartilage cells. The transverse osseous plate underneath the hypertrophic cartilage was thickened as compared with that at the end of 1 month of injection, but resorption of the bony material as well as of the calcified and partly ossified plugs had also made progress.

However, even after 3 months of the treatment there were only small perforations of the epiphyseal zone, and no extensive epiphyseo-diaphyseal union had occurred. No alterations of the articular cartilage were noted. In the bony shaft, increased vascular and cellular resorption had caused a removal of the excess bone found at the earlier stages of the experiment. In some instances this resorption was so marked that the compacta appeared even thinner than in untreated animals of corresponding age.

Mice of Strain C_{57} 3 Months Old at the Beginning of the Experiment, and Approaching the End of the Growth Period

One week after the beginning of the injections of Antuitrin G the width of the epiphyseal growth zone did not differ greatly from that in corresponding noninjected mice. The number of cartilage cells in a single cartilage cell row was not increased and there was no evidence of a stimulation of the proliferation of the cartilage cells as seen in younger animals at the corresponding stage of the experiment. However, many of the more proximally situated cartilage cells had assumed a vesicular appearance, indicating that these cells had undergone an increased hypertrophy. The layer of the hypertrophic cartilage cells proper was narrower than usual due to a rapid ossification. Because of increase in the size of the cells, the cartilaginous ground substance appeared diminished. Hand in hand with the increased hypertrophy of the cartilage cells went regressive changes, leading to the destruction of cartilage cell rows and to the appearance of fairly thick, amorphous plugs of degenerated cartilage in the epiphyseal plate. After 2 weeks of injections, there was likewise no noticeable stimulation of cell multiplication in the epiphyseal cartilage, but regressive changes of the latter were intensified (Figs. 3 and 4). The cartilaginous matrix and the cartilage cells were increasingly calcified and the ossification of the hypertrophic cartilage was accelerated. The amorphous plugs in the epiphyseal disk were thicker than after 1 week of treatment, and had undergone further calcification and replacement by bone. Vascular and cellular resorption became more and more pronounced, causing a dissolution of the osseous plugs and of the osseous spicules to such a degree that in some places only a thin bony plate separated the epiphyseal cartilage from the diaphyseal marrow. Similar changes produced a thinning of the bony border lamella separating the articular cartilage from the epiphyseal marrow. The cartilage cells of the joint showed hyperplastic and hypertrophic changes and underwent regression. The cartilaginous ground substance was softened and fibrillar; but no severe arthropathic lesions were produced (Figs. 5 and 6). The peri-

osteal tissue was loose, and increased vascular and osteoclastic resorption led to a thinning of the bony shaft.

After 4 weeks of treatment, the conditions were similar to those seen after 2 weeks, but, on the whole, the resorptive processes were more marked than the degenerative changes of the cartilage.

Mice of Strain D, 5 to 6 Weeks Old at the Beginning of the Experiment

As reported previously,[10] the skeletal tissues of mice of strain D exhibit normally a faster rate of skeletal ageing than those of strain C_{57}. Thus in untreated mice 8 weeks old of strain D, the growth zones appeared only slightly younger than those of a mouse 4 months old of strain C_{57}.

After 1 week of administration of Antuitrin G, there was somewhat increased proliferation of the epiphyseal cartilage, which, however was less accentuated than in corresponding mice of strain C_{57}. As compared with the latter, the tendency of the cartilage cells of mice of strain D to undergo hypertrophy, retrogression and ossification was likewise less intense, but these processes were still increased over the normal.

After 2 weeks of injections, proliferation of the cartilage cells had increased slightly or not at all, whereas hypertrophy and degeneration of these cells had progressed further. After 4 weeks of injections, the retrogressive changes in the cartilage and the formation and resorption of bone were slightly advanced over the normal; in isolated areas, perforations of the epiphyseal disk were initiated.

Mice of Strain D 3 Months Old at the Beginning of the Experiment

One and 2 weeks subsequent to the beginning of the administration of Antuitrin G there was no evidence of a stimulation of the proliferation of the epiphyseal cartilage, that normally at this age is already in an inactive state. But the regressive changes taking place in the cartilage were somewhat advanced over the normal. Resorptive processes acting on cartilage and bone were likewise intensified. After 4 weeks of treatment in mice of strain D, that were then 4 months old, advanced resorptive processes had led to fairly wide perforations of the epiphyseal disk. This may seem at first to be a more marked effect than that exerted by Antuitrin G in corresponding mice of strain C_{57}, in which only small perforations of the epiphyseal zone were present, but the condition produced in mice of the D strain was comparable to that seen in normal mice of this strain at the age of 6 to 7 months, and thus the structural age of the cartilage was increased by about 2 to 3 months in these mice. In mice of strain C_{57}, on the other hand, the structure of the epiphyseal zone in animals 4 months old treated with

Antuitrin G for 4 weeks corresponded to that seen in normal mice of this strain at the age of 10 to 12 months. In mice of strain C_{57}, the structural age of the epiphyseal cartilage was thus advanced by about 6 to 8 months, which is about three times as much as in mice of strain D.

Even in littermates, treated or untreated, individual differences as to the progress of skeletal ageing and of the weight curves exist.

Guinea Pigs

After 4 days of injections of Antuitrin G, the epiphyseal growth zone of the upper tibia in guinea pigs was somewhat narrower than usual. The number of cartilage cells in a single cell column was decreased as compared with the normal. The cartilaginous ground substance was swollen and vacuolated. The columnar cartilage cells had undergone a marked mitotic proliferation and rapid hypertrophy, and, as was the case in young mice, the zone where the hypertrophy of the cartilage cells began was shifted farther proximally. The largest cartilage cells of the hypertrophic type present were smaller than usual, the largest ones being replaced by bone as soon as they had been formed. In the subepiphyseal layer, instead of a richly vascularized hemopoietic marrow, a cellular connective tissue had developed in which numerous mitoses indicated the degree of stimulation exerted on the connective tissue cells. A great number of these cells had been converted into mitotically proliferating osteoblasts, whereas others participated in resorptive processes which were intensified. In these two antagonistic processes the former was predominant for there was an increase in the number of trabeculae. The articular cartilage cells were likewise hyperplastic and hypertrophic. There was a marked increase of cells in the periosteum.

Two weeks subsequent to the beginning of the administration of Antuitrin G, the proliferation of the epiphyseal cartilage was still somewhat intensified. However, in the meantime, regressive and resorptive processes had also made progress and had begun to predominate over the growth processes. Ossification had likewise markedly progressed. Because of the increase in formation as well as in resorption of bone, the subepiphyseal tissue showed an irregular appearance. In some places, fibrous tissue had replaced the excessive number of trabeculae found previously and had penetrated through the cartilaginous disk into the epiphyseal marrow cavity. In other areas, strands and islets of unopened columnar cartilage cell capsules were lying far distally in the metaphysis, enclosed by osseous tissue.

In the articular cartilage, hyperplastic and hypertrophic processes were associated with increased resorption leading to a thinning and

corrosion of the bony border lamella separating the epiphyseal cavity from the articular cartilage. The cells at the inner and outer surface of the compacta of the shaft proliferated. The vascular canals were enlarged, and the resorption of bone began to become conspicuous. At the chondro-osseous junction of the ribs the changes observed were similar to those at the growth zones of tibia and femur.

Comment

Antuitrin G administered subcutaneously to growing mice and guinea pigs increased temporarily the proliferation of the growing cartilage, accelerated its hypertrophy and caused a premature onset and an acceleration of degenerative and resorptive processes in the epiphyseal growth zones. Formation of bone in the subepiphyseal tissues and in the shaft was temporarily increased, but this increase was followed by enhanced processes of resorption. Antuitrin G also called forth hyperplastic, hypertrophic and slight regressive changes in the articular cartilage but it did not produce severe arthropathic lesions.

In adult mice, 3 to 4 months old, in which the growth capacity of the epiphyseal cartilage is almost exhausted, Antuitrin G failed to induce any proliferation in the epiphyseal cartilage. However, the columnar cartilage cells, which were for the greater part preserved at the beginning of the treatment, underwent an accelerated degeneration and calcification followed by breakdown and ossification. In the subepiphyseal tissues and in the shaft, resorption of bone became prominent. In the joint, hyperplastic and hypertrophic changes of the cartilage were associated with regressive processes, but likewise without the occurrence of severe lesions.

Antuitrin G is more effective in accelerating the skeletal age changes in mice of strain C_{57}, which normally show a slow rate of skeletal ageing, than in mice of strain D, the skeleton of which ages more rapidly under normal conditions.[10] On the whole, therefore, Antuitrin G exerts on the skeleton effects similar to those exerted by acid extracts of the bovine anterior hypophysis formerly used by us, although, in the dose given, Antuitrin G had a weaker effect on the skeleton of mice than corresponding amounts of the bovine anterior pituitary extracts.

In immature guinea pigs, both Antuitrin G and acid anterior hypophyseal extracts are more effective on the skeletal tissues than in mice. Both substances stimulate temporarily the proliferation of the epiphyseal and articular cartilage and cause a premature onset of degeneration and an acceleration of ossification. Apparently, however, Antuitrin G intensifies resorptive processes more than does acid an-

terior hypophyseal extract. Whereas perforations of the epiphyseal disk could be initiated after 2 weeks of injections of Antuitrin G, even under prolonged treatment with anterior hypophyseal extract increased amounts of bone could still be present in the diaphysis and in the beaded chondro-osseous junction of the ribs.[3,11] This delay of the progress of resorptive processes may, however, be correlated with the decrease of effectiveness of the acid hypophyseal extract in the course of prolonged administration.[3] Under certain conditions that are now being investigated, resorptive processes are likewise resumed in guinea pigs under the influence of acid anterior hypophyseal extract, leading to a removal of the excessive amounts of bone.

Antuitrin G as well as acid anterior hypophyseal extract may accelerate every phase of skeletal growth and ageing, the phases affected in each single instance being those that are in progress at the beginning of the treatment. In other words, Antuitrin G, like other hormones, alters the time curve of the tissues on which it acts (Loeb[12]). No evidence could be obtained from the present investigations that in normal animals Antuitrin G stimulates the growth of the cartilage beyond its normal limits. In growing animals the temporary increase of proliferation is balanced by a shortening of the period during which growth takes place, and results in an accelerated exhaustion of the growth capacity of the cartilage. In animals in which the growth capacity of the cartilage has been naturally exhausted, no resumption of proliferation of cartilage could be effected by the administration of Antuitrin G. The temporary character of the growth stimulation of the epiphyseal cartilage as well as the lack of growth stimulation in adult animals explain the failure of this substance to produce gigantism.

Growing mice receiving Antuitrin G did not weigh more than did the control mice and in many instances weighed less. The guinea pigs treated with Antuitrin G showed an initial weight loss. A greater gain in weight of the injected mice as compared with their controls became noticeable only after the growth period was terminated. This occurred in animals in which the growth period had ended prematurely due to the injections of Antuitrin G as well as in animals which had reached the end of the growth period, when the treatment was begun. In the latter case, the increase of weight began immediately after the first injection and might have been largely due to storage of fat (Evans and Long[13]) or to an accumulation of water by the tissues (Downs[14]).

The difference in the response of the weights of growing and adult mice might be connected with the different reactions of the thyroid gland to Antuitrin G at different ages. Loeb[15] studied the thyroid glands of the mice and guinea pigs used in these investigations and

found in the growing animals, particularly in the guinea pigs, a marked thyrotropic effect of Antuitrin G, similar to that seen after administration of acid anterior hypophyseal extract of cattle; in guinea pigs this effect decreased with increasing duration of the treatment. Loeb and his associates [5,16,17] had furthermore shown that there was no essential difference in the effects of acid and alkaline extracts of the anterior hypophysis of cattle and sheep on the thyroid and ovary of the guinea pig.

There is a fundamental similarity in the histologic changes produced in the skeleton by the acid anterior hypophyseal extract and Antuitrin G. This similarity is of interest because it had been suggested by some investigators [7,8] that the acid anterior hypophyseal extract used by us in previous experiments was too crude for the demonstration of the action of the so-called growth hormone. Furthermore, the result obtained with the extract should not be comparable to those produced by the alkaline extract of anterior hypophysis.[9,18] However, recently, Ray, Evans and Becks,[6] after injecting purified alkaline hypophyseal extracts in rats for 10 days, reported a marked stimulation of endochondral ossification, atrophy of trabeculae and a less marked periosteal ossification. These histologic findings agree essentially with those we have previously observed at certain stages following injections of acid extracts of bovine anterior hypophysis; these changes are interpreted as part of the general acceleration of growth and ageing processes of the skeletal tissues. As to possible effects on the skeletal tissues of the gonadotropic fraction contained in the acid extract and which Ray, Evans and Becks believed to be active in producing our results, we may refer to one of our previous investigations.[2] In that study we investigated the influence of implants of the bovine anterior hypophysis on the epiphyseal cartilage of guinea pigs. One group of animals received the fresh glands; others received glands that prior to implantation had been treated in such a way as to suppress one or the other gonadotropic activity (Loeb and Hayward [19]). In experiments conducted as long as 6 days we were unable to establish any correlation between the reaction of the epiphyseal cartilage to the follicle-stimulating or luteinizing, or atresia-producing action of such implants.

The lack of any significance for these gonadotropic fractions in inducing an indirect effect on the epiphyseal cartilage is also indicated by the fact that anterior hypophyseal extract exerts its influence also in castrated animals.[20,21] Furthermore, the predominant gonadotropic fraction contained in the acid extract being the luteinizing hormone, a secondary effect which might be exerted by the intermediation of the ovaries should be similar to that of progesterone. We [22] were able

to show that the latter hormone causes a delay in the ageing of the skeleton which is the opposite of the effect called forth by the acid hypophyseal extract.

Ross and McLean [9] found that Antuitrin G, injected intraperitoneally into rats 6 months of age, had no effect on the growth zones. The lack of proliferation of the epiphyseal cartilage in their rats corresponds to our findings in mice, which were 3 months old at the beginning of the treatment. In these mice we found no effect of Antuitrin G on the proliferation of the cartilage, but there occurred increased ageing of the skeletal tissues. It is, of course, possible that the ineffectiveness of Antuitrin G in rats in the experiments of Ross and McLean might have been due to species differences.

According to our findings, Antuitrin G may be included in the group of those hormones or hormonelike substances which, under certain conditions, can accelerate all three phases of skeletal growth and ageing; in this group belong also the anterior hypophyseal extracts,[4] thyroxin [23] and potassium iodide.[24] All of these substances [25] influence in a similar way the growth and ageing of the epiphyseal cartilage of normal animals. It seems doubtful, therefore, whether one is justified in stipulating that there is a single specific growth hormone produced by the anterior hypophysis, inasmuch as processes other than those of growth are affected by the same substances.

Summary

In growing mice and guinea pigs, Antuitrin G influences all three phases of skeletal growth and ageing. It causes a temporary stimulation of the proliferation of the epiphyseal cartilage, associated with a shortening of the growth period and followed by premature onset and an accelerated course of age changes such as degeneration, calcification and breakdown of the cartilage. Antuitrin G also promotes processes of bone formation as well as those of resorption of bone.

In adult mice which have reached the end of their physiologic growth period, Antuitrin G fails to call forth renewed proliferation of the epiphyseal cartilage. But such processes as are in progress at the time of the beginning of the experiment are accelerated under the influence of Antuitrin G.

The effects of Antuitrin G on cartilage and bone are, therefore, in principle, the same as those exerted on these tissues by acid anterior hypophyseal extract; the differences that do exist largely concern the degree of the changes produced, the anterior bovine hypophyseal extract being more effective in mice. Antuitrin G may thus be included in one group with various other anterior hypophyseal extracts, thy-

roxin and potassium iodide, all of which influence in a similar way the growth and ageing processes in the skeletal tissues.

NOTE: We are indebted to Dr. Oliver Kamm of Parke, Davis & Co. for the Antuitrin G used in these experiments, and to Edgar Nebel for the photomicrographs.

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DESCRIPTION OF PLATES

PLATE 171

FIG. 1. Section through the epiphyseal disk of the upper tibia of a normal male mouse of strain C_{57}, 7 weeks old, whose weight had increased from 14.5 to 19.0 gm. during 1 week of observation. × 125.

FIG. 2. Section through the epiphyseal disk of the upper tibia of a male littermate of the animal shown in Figure 1, which had been injected with 1 rat unit (0.1 cc.) of Antuitrin G three times weekly for 1 week. The weight had increased from 14.5 to 16.3 gm. Ossification of the epiphyseal cartilage has progressed farther than in Figure 1. There is a plug of degenerated cartilage in the epiphyseal plate. × 125.

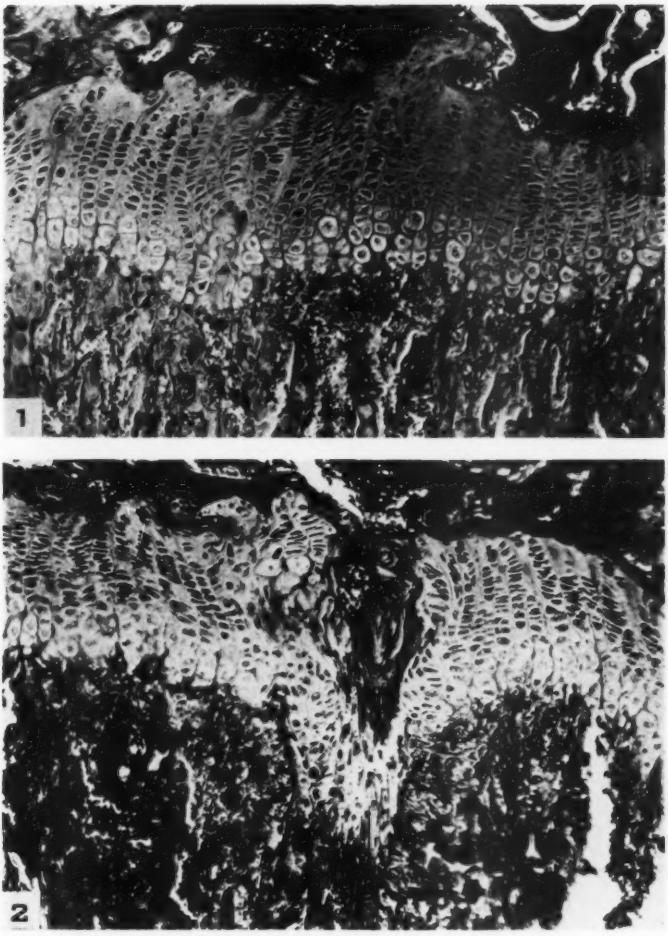
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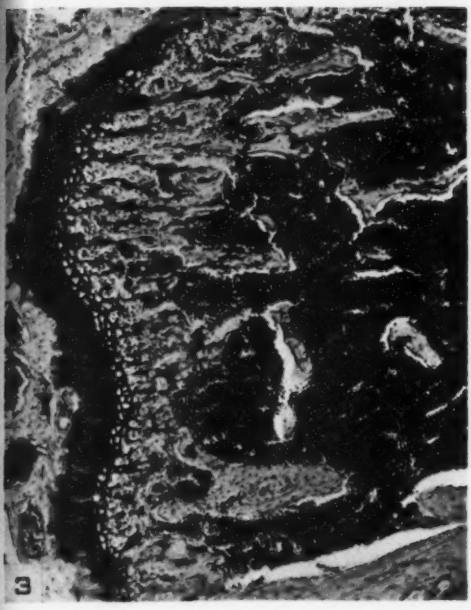
PLATE 172

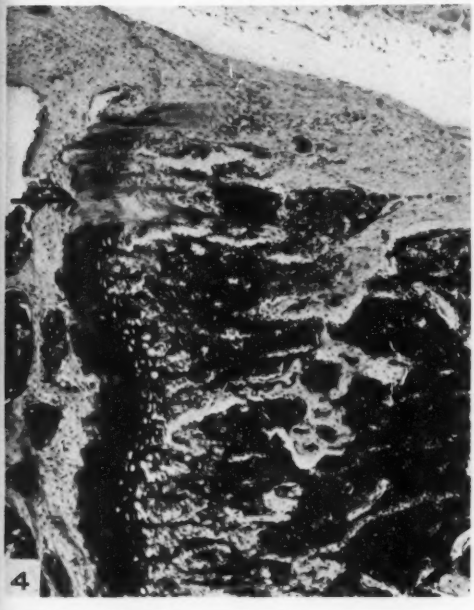
FIG. 3. Section through the epiphyseal disk of the upper tibia of a normal male mouse of strain C_{57}, 16 weeks old; initial and final weights were 20.5 gm. for 2 weeks of observation. × 80.

FIG. 4. Section through the epiphyseal disk of the upper tibia of a male littermate of the animal shown in Figure 3, which had been injected with 1.5 rat units (0.15 cc.) of Antuitrin G three times weekly for 2 weeks. The weight had increased from 21.5 to 24.4 gm. during this period. Resorption of cartilage and of subepiphyseal trabeculae has advanced farther than in Figure 3. There is an ossified plug at the right margin of the epiphyseal plate. × 80.

FIG. 5. Section through the articular surface of the lower femur of the same animal as shown in Figure 3. × 230.

FIG. 6. Section through the articular surface of the lower femur of the same animal shown in Figure 4. Increased hyperplasia and hypertrophy of the articular cartilage and retrogressive changes of cartilaginous matrix and of some cartilage cells. × 230.





Silberberg and Silberberg Skeletal Effects of Antuitrin G

FIBROSIS OF THE PANCREAS IN MEAT-FED DUCKS*

I. Arthur Mirsky, M.D., Samuel Elgart, M.D., Norton Nelson, Ph.D., and Philip Wasserman, M.D.

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Pancreatectomy of domesticated ducks,[1] unlike that of owls,[2] does not result in the development of permanent hyperglycemia. The essentially herbivorous nature of the duck (in contrast to the carnivorous habits of the owl) made a study of the relation of the diet to the response to pancreatectomy of interest.

Beginning at 1 day of age, ducks were fed freely on a diet of raw ground meat (50 cc. of cod liver oil was added to each 8 lbs. of meat). Birds that attained an age of 6 months to 1½ years were used for this study. Each of these was depancreatized and subsequently its diabetic status was evaluated. A total of 30 birds was thus studied.

The majority of the ducklings took the diet voraciously. During the ensuing months many of the meat-fed birds developed marked edema of the extremities, periarticular fibrosis, arthritic changes and calcareous deposits about the joints. The bills and webs became white. Administration of carotene had no effect on this deficiency in pigmentation.

In none of the 30 animals that were depancreatized did a permanent hyperglycemia ensue. However, in most instances, a marked hyperglycemia was noted for 2 to 4 days after the operation, reaching levels as high as 900 mg. per cent in some birds. Following this, the blood-sugar level returned to normal or the animal died within 4 days with hyperglycemia.

Animals operated upon at the age of 6 to 7 months revealed grossly and microscopically normal pancreatic tissue. In animals that had been on the diet for periods longer than 7 months, the pancreas was small and difficult to remove. It felt cartilaginous, and cut like cartilage or like markedly fibrotic tissue. Histologic sections of these pancreases revealed varying degrees of fibrosis ranging from slight patchy change to almost complete replacement of normal structure by a hyperplastic connective tissue resembling a neoplasm. In the pancreases of nearly all ducks on the diet for longer periods (8 to 18 months) fibrosis predominated, whereas in those from animals on the diet for short periods (6 to 8 months) relatively little abnormality was noted. The normal pancreas of the duck is illustrated in Figure 1. Typical changes that occur in meat-fed ducks are depicted in Figures 2, 3 and 4.

* Received for publication, March 5, 1942.

It is obviously not feasible to attempt to explain the mechanism involved in the production of the change noted above, beyond noting that the diet of meat with cod liver oil was deficient in a large number of dietary essentials. It is quite possible that some food factor may be necessary for the maintenance of the normal pancreas, or it may be that a meat diet induces first a stimulation and subsequently an exhaustion with atrophy and fibrosis of the pancreatic acini. Further studies to elucidate the mechanism responsible for the above mentioned changes are in progress.

Summary

Ducks maintained on a diet of meat and cod liver oil develop pancreatic fibrosis that apparently is progressive. The first changes are seen in about 8 months.

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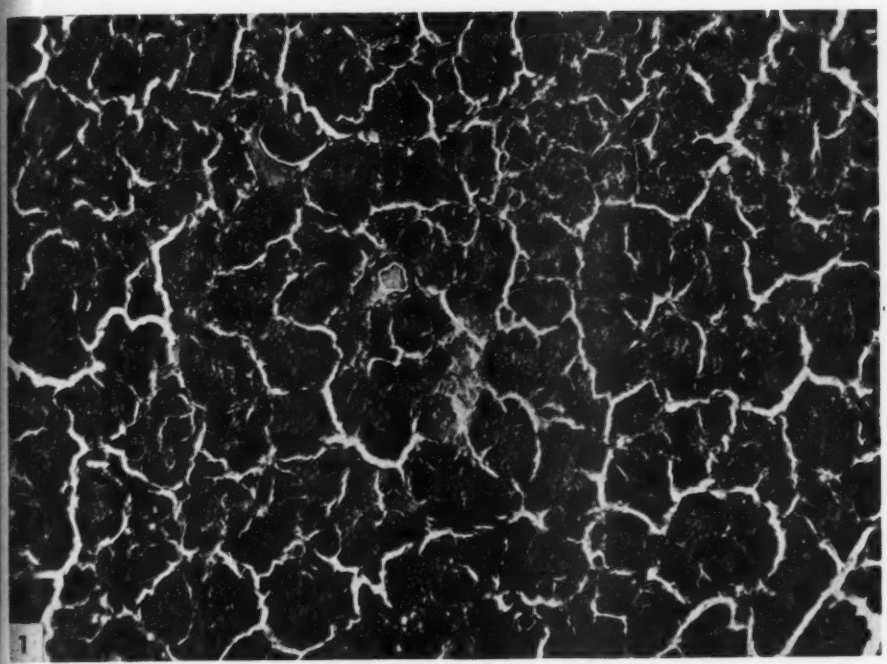
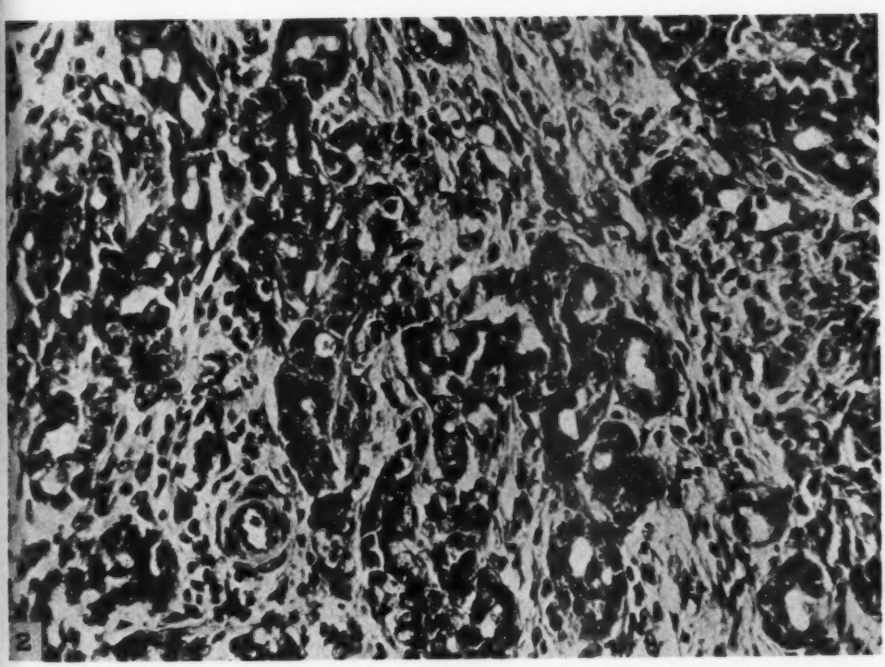
Plate 173

Fig. 1. Pancreas of a duck fed a normal herbivorous diet. Well preserved acinar tissue is seen. There is an occasional group of light-staining cells suggesting islet tissue.

Fig. 2. Pancreas of a duck fed meat for 6 months. Histologic detail is confused, the acini appearing somewhat irregular in size and shape. There is a distinct fibrosis with strands of connective tissue separating acini and somewhat distorting them. Some of the cells appear frayed and form enlarged glandular elements with papillalike structures projecting into the lumen.

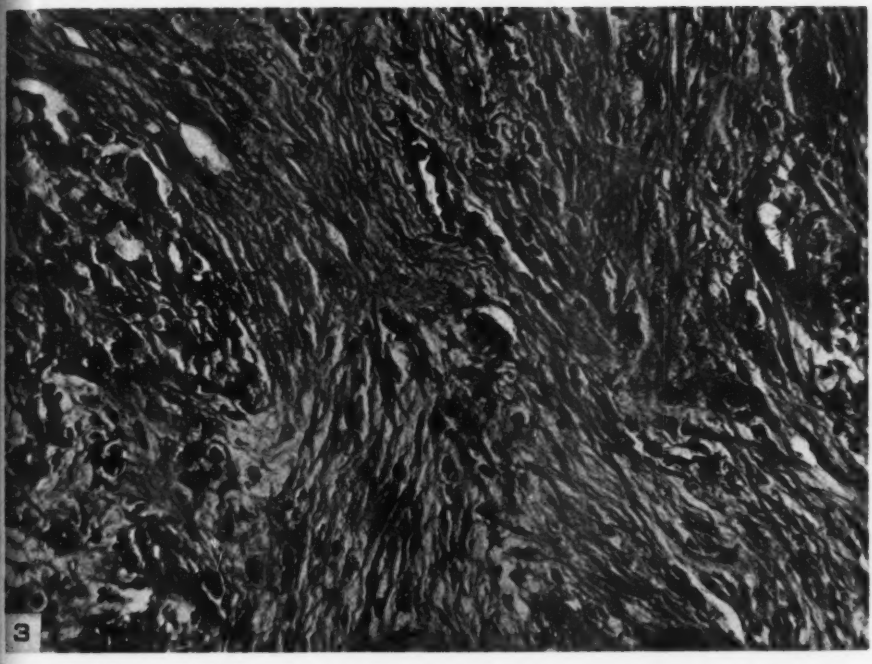




Mirsky, Elgart, Nelson and Wasserman Fibrosis of the Pancreas

PLATE 174

FIGS. 3 and 4. Pancreas of a duck fed meat for 16 months. There are areas where complete replacement fibrosis leaves no acinar or epithelial structures. The fibrosis is compact and relatively acellular. In isolated areas are found groups of epithelial cells in rounded nests surrounded by abundant fibrous tissue. The epithelium here is not in the acinar arrangement nor is it suggestive of islet tissue. There are areas which appear pseudoneoplastic in that the fibrous tissue presents a sweeping configuration in regions exhibiting the tendency to complete obliteration of the epithelial elements. No islet tissue is identifiable.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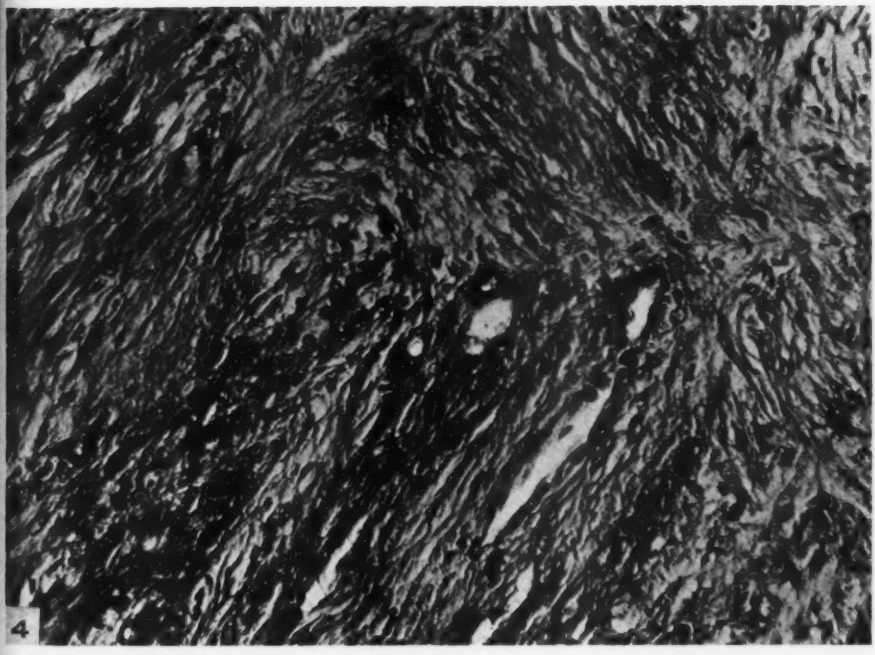
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